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Rehabilitation of aquatic invertebrate communities
in raised bog landscapes

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Rehabilitation of aquatic invertebrate communities in raised bog landscapes

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Chapter 1

Introduction

Gert-Jan van Duinen

◀ The peat moss *Sphagnum cuspidatum* and common cottongrass *Eriophorum angustifolium* growing in a lagoon created by black peat bunds in the bog remnant Bargerveen, The Netherlands.

1.1 Characteristics of raised bog landscapes

Peatlands are considered as one of the most important natural ecosystems of the world, because of their key value for biodiversity, regulation of climate and water supply, and important support for human welfare. However, the degradation of peatlands is ongoing in many parts of the world. This is due to cultivation, drainage, afforestation, peat extraction, increased atmospheric deposition of nitrogen and sulphur, and climate change. In Europe the total area of still accumulating peat is estimated to be less than 50 % of the former peatland area. In Germany and The Netherlands this percentage is even 1 % or less. Therefore, global priority should be given to the protection, restoration and wise use of peatlands (Joosten & Clarke 2002, Parish et al. 2008).

The accumulation and storage of peat is an essential feature of peatlands. This feature depends on a water level close to the surface and waterlogging of the peat deposit, reducing the rate of decay of organic material to an extent that it is slower than its production rate (Joosten & Clarke 2002, Parish et al. 2008). Peatlands where peat is currently forming and accumulating are called mires. Mires are generally divided into bogs and fens. Bogs typically have peat deposits raised above the surrounding landscape, receiving water, minerals and nutrients only by precipitation (therefore called ombrogenous or ombrotrophic mires), whereas fens receive inflow of groundwater or surface water which has been in contact with mineral soil (therefore called geogenous or minerotrophic mires). Consequently, bogs are characterised by a low mineral and nutrient availability and are only present in regions with a surplus of precipitation (Gore 1983, Joosten & Clarke 2002). Peat mosses (*Sphagnum* spp.) are keystone species in the functioning of bogs. They create their own acidic and moist environment and play a key role in peat accumulation, development of the surface microtopography (especially hummocks and hollows), and the hydrological self-regulation of bog ecosystems (Van Breemen 1995, Rochefort 2000).

Raised bogs are the principal class of *Sphagnum* bogs in the boreal and cool temperate zone. They typically form a dome-shaped bog massif and have a surface pattern of microtopes: hummocks, hollows, lawns, ridges, and pools. The cross-sectional shape of the dome and the microtopography of the raised bog surface vary depending on the climatic conditions. These include air temperature, precipitation and its distribution over the year, and evapotranspiration (Gore 1983, Lindsay et al. 1988). These climatic conditions affect processes involved in natural self-organisation in bog ecosystems including mineralisation, lateral water flow, and intraspecific competition between *Sphagnum* and vascular plant species (Couwenberg & Joosten 2005, Eppinga et al. 2007).

Raised bog massifs are embedded in the wider landscape (Figure 1a). Therefore, raised bog landscapes are characterised not only by the ombrotrophic, extremely nutrient limited and acid bog massif mainly formed by *Sphagnum* mosses, but also by gradients from the bog massif to the more minerotrophic surroundings, where the harsh conditions of the bog massif are gradually offset (Wheeler & Proctor 2000). The conditions at the interfaces of bog margins with the adjoining mineral soil are shaped by the character of both the surrounding landscape and the bog massif, including the steepness of the marginal slope of the bog massif (called 'rand'; Wheeler & Shaw 1995). Depending on the topography of the landscape and mineral soil type a narrow or more extensive transitional mire, fen, or swamp is present (Howie & Tromp-van Meerveld 2011). The wet margin around raised bogs is called lagg (Osvald 1933). This term is more recently used for various sharp or diffuse transitional zones receiving water from both the

bog massif and the surrounding mineral soil (Howie & Tromp-van Meerveld 2011). Many raised bog landscapes consist of a complex of multiple bog massifs and sometimes include rivulets or mineral soil 'islands' partly or completely surrounded by mires. The variation in environmental conditions within mire landscapes can be described by various environmental gradients, including gradients in acidity and availability of minerals and nutrients (Sjörs 1948, Bridgham et al. 1996, Wheeler & Proctor 2000). These gradients can be identified at different spatial scales. A gradient of poor to rich in nutrients and minerals is present between the ombrotrophic centre of the bog massif and the lagg or fen at its margin. Gradients of wet to dry are present within hummock-hollow complexes and may be found between the wet centre of a bog massif, its relatively dry margin, and a wet lagg.

Spatial scales in raised bog landscapes

The identification of different organisational levels of mire ecosystems and corresponding spatial scales proved to be practical in the investigation, conservation, and restoration of raised bogs and other types of mires (Table 1; Sjörs 1948, Lindsay et al. 1988, Couwenberg & Joosten 1999, Schouwenaars et al. 2002, Schouten 2002). A complex of two or more hydrologically interdependent mire massifs is called a macrotope. Schouten (2002) described the macro-scale as the scale level that comprises the bog and the wider landscape in which the peatland is embedded (Figure 1A), also referred to as 'bog landscape'. Consequently, Schouten (2002) considers the bog, lagg and surrounding mineral soil system as different ecological systems at meso-scale. A single raised bog massif is identified as mesotope. A microtope is defined as a characteristic arrangement and combination of surface features (microforms) within the mire massif (e.g., hummock-hollow complex, or the arrangement of ridges and pools, corresponding to the community-complex level identified by Schouten 2002). The individual features within the surface patterning of a mire (e.g., a single hummock, hollow, or pool) are termed microforms, or nanotope. This level corresponds to the micro-scale identified by Schouten (2002).

Table 1. Organisational levels and spatial scales in mire ecosystems.

Name of level (Couwenberg & Joosten 1999)	Synonyms	Indication of size (m ²)	Scale (Schouten 2002)	Example
Nanotope	Mire micro-form, feature	10 ⁻¹ -10 ¹	Micro-scale	Hummock, hollow, pool
Microtope	Mire site	10 ⁴ -10 ⁶	(community- complex)	Hummock-hollow complex, pool-ridge complex
Mesotope	Mire massif	10 ⁵ -10 ⁷	Meso-scale	Raised bog, lagg, surrounding mineral soil system
Macrotope	Mire system, mire complex	10 ⁷ -10 ⁹	Macro-scale	Bog landscape, including wider landscape

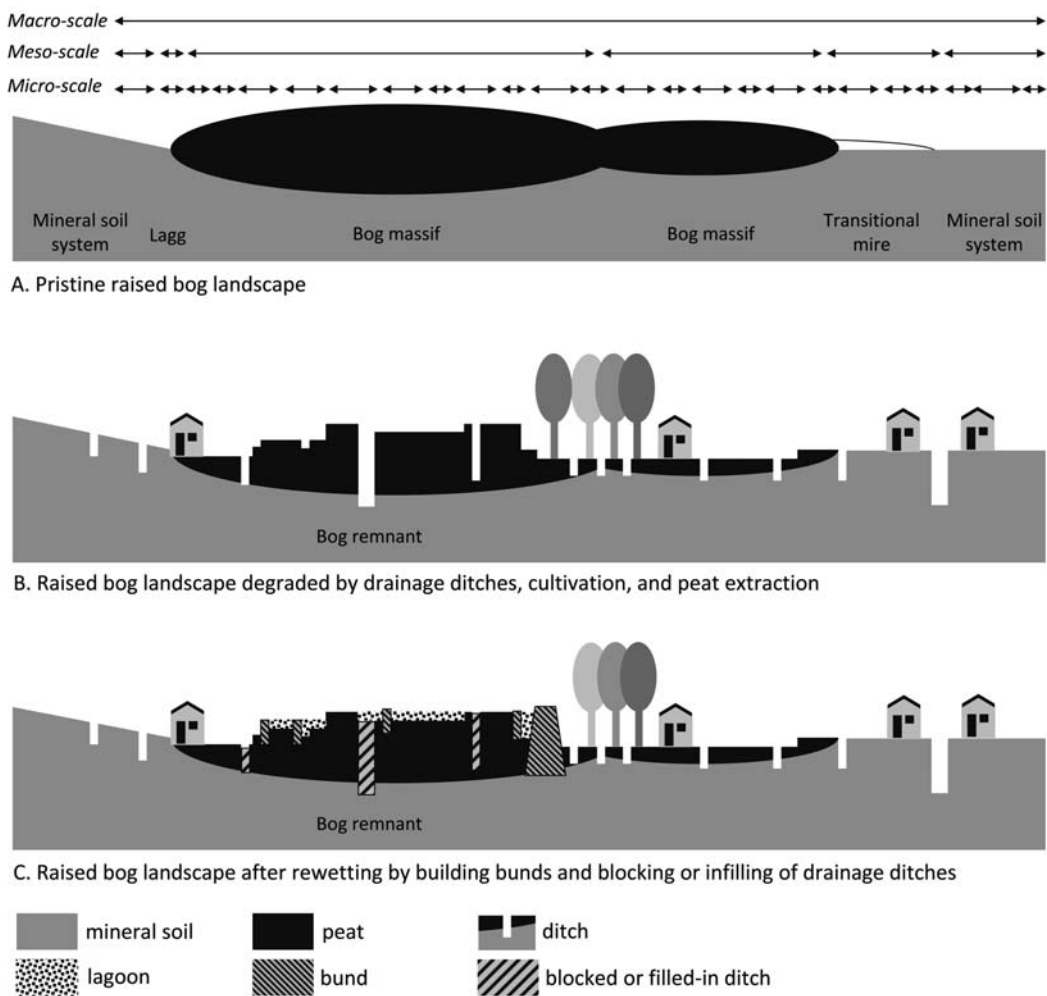


Figure 1. Schematic representation of pristine raised bog landscapes (A) and the situation after degradation (B) and implementation of restoration measures (C). Spatial scales identified by Schouten (2002) are indicated above scheme A, with each section of the lines representing a unit at the respective spatial scale levels.

1.2 Fauna and habitat diversity in raised bog landscapes

Fauna species assemblages vary along the gradient across raised bog massifs with highest numbers of species and individuals at the marginal slope and lagg (Väisänen 1992). Because of their acidity, extremely low nutrient-availability, and wetness, the ombrotrophic parts of raised bog landscapes are hostile habitats for many plant and animal species. *Sphagnum* bogs are species-poor relative to several other biotopes, both in regard to vertebrates and invertebrates (Harnisch 1925, Peus 1932, Bölscher 1988, Spitzer & Danks 2006). Chemical deficiencies and low pH almost entirely exclude some taxonomic groups e.g., lumbricid worms, isopods, and shelled snails from bog massifs (Harnisch 1925, Peus 1932, Burmeister 1980, Speight & Blackith 1983) and low nutrient-availability may exclude animal species that have high nutrient demands.

The proportion of characteristic species in bogs is quite high, compared to other biotopes, because of the special environmental conditions (Maavara 1955, Spitzer & Danks 2006). Both characteristic and tyrphoneutral species (Table 2) might depend on a combination of environmental conditions provided by the gradient between the ombrotrophic centre of the bog massif, the marginal slope, the lagg, and surrounding biotopes to complete their life cycle or for persistence of the population (Moller Pillot 2003, Verberk et al. 2006). For instance, Golden Plover (*Pluvialis apricaria*) and Black Grouse (*Lyrurus tetrix*) exploit bog landscapes at the mesotope or macrotope scale, breeding in the central raised bog and foraging in the more nutrient and mineral rich edges of the bog landscape (Niewold 1993, 1996, Heckenroth 1994). Also the internal diversity in site conditions at the scale of the bog massif is used by species that need different sites to complete their life cycle. For instance, carabid beetles operating on the microtope scale, inhabiting the open bog plateau, stay in the top of dry hummocks in winter and forage in damp *Sphagnum* lawns in summer (Främbis 1994). Species assemblages of arthropods operating on the scale of the nanotope differ considerably between dry hummocks and wet hollows in raised bogs (Burmeister 1980). Microfauna assemblages even show variation at a spatial scale of several centimetres within a macroscopically homogeneous *Sphagnum* carpet, as was shown for testate amoebae (Mitchell et al. 2000). Thus, the diversity in environmental conditions at various spatial scales is a prerequisite for hosting the full species diversity of raised bog landscapes.

Categories of species characterizing the raised bog fauna

Four categories of species were used by Peus (1932) to characterize the fauna of raised bogs (Table 2). Considering the acidity of bogs, the terms acidobiontic and acidophilous (Hustedt 1939, Drost et al. 1992) can also be used here to classify species in respect to their pH preference (Table 2). Most of the species living in bogs, including a number of abundant species, can also live outside bogs and can be characterized as tyrphoneutrals or generalists, i.e. species occurring in various types of biotopes (Maavara 1955, Danks & Rosenberg 1987, Runtz & Peck 1994). Maavara (1955) found about 800 insect and spider species in Estonian bogs and Valk (1988) gave the number of 1,200 insect species of which 49 are in Estonia only found in bogs. Eighty percent of the spider species present in North and Central European bogs (including the four most abundant species) can be regarded as generalist species (Schikora 2002), whereas in Canadian bogs 5.6 % of the spider species found was classified as tyrphobiontic and 10 % as tyrphophilous (Dondale & Redner 1994). Still, tyrphoneutral species and even invertebrates blown into the bog from the surrounding landscape are part of the bog community and foodweb (Reynolds 1990).

Table 2. Categories of species used to characterize the fauna of raised bogs.

Category	Definition	References
Tyrphobiontic	Species occurring only in bogs	Peus (1932), Spitzer & Danks (2006)
Tyrphophilous	Species characteristic of bogs but not confined to them	
Tyrphoneutral	Species resident in bogs but also occurring in other habitats	
Tyrphoxenous	Nonresident vagrants or erratics that cannot live in bogs	
Acidobiontic	Species mainly occurring at pH<5.5	Hustedt (1939), Drost et al. (1992)
Acidophilous	Species mainly occurring at pH<7	

1.3 Effects of degradation of raised bog landscapes

Pristine raised bog landscapes are nowadays only present in remote areas. In more densely populated areas in Western Europe and certain areas in temperate North America raised bog landscapes have almost completely disappeared (Poulin & Pellerin 2001, Joosten & Clarke 2002). Here, remaining bogs, including bog remnants under conservation or restoration at present, are damaged to varying degrees (Barkman 1992, Wheeler & Shaw 1995, Schouten 2002, Vasander et al. 2003). Digging of watercourses and drainage ditches in the mineral soil, in which raised bog complexes are embedded, started already centuries ago in parts of Western Europe (Baaijens et al. 2011a, 2011b). This likely damaged biotopes that were vulnerable to human interference in local and regional hydrology, like lagg zones and other biotopes fed by groundwater within bog landscapes. Cultivation, peat cutting, and afforestation of bogs, preceded by drainage, started at the edge of bog landscapes (Van den Munckhof 1993, Wheeler & Shaw 1995, Laine et al. 2006). As a result, lagg zones and other transitional biotopes between bog massifs and the surrounding landscape have been destroyed or damaged (Figure 1b), except some in remote areas or in exceptional landscape settings. This loss has likely caused the decline of plant and animal species dependent on these transitions.

In past centuries in Western Europe cultivation and peat cutting by hand power gradually proceeded towards the centre of bog landscapes (Van den Munckhof 1993). Thus, most bog remnants under conservation or restoration nowadays are remnants of former ombrotrophic bog massifs. Burning of bogs for the cultivation of buckwheat (*Fagopyrum esculentum*) caused large losses of peat in the past (Göttlich & Kuntze 1980, Van der Schaaf 1999). The pattern of trenches dug for superficial drainage is still visible in several bog remnants. The irregular small-scale peat cutting by individual farmers resulted in a mosaic of water filled peat holes and dry strips of peat, still present in several bog remnants in Western Europe (Barkman 1992, Wheeler & Shaw 1995). After abandonment, secondary succession occurred. This resulted in a mosaic of *Betula* wood, heathland vegetation with *Calluna vulgaris*, *Erica tetralix*, and *Molinia caerulea*, and peat holes with open water or with various types of *Sphagnum*-dominated vegetation (Barkman 1992). In such degraded bogs several characteristic bog species are still present, but species that do not occur at ombrotrophic conditions in pristine *Sphagnum* bogs (i.e., wet, acid, nutrient-poor) have been able to establish in bog remnants (Koponen 1979, Burmeister 1980, Främbis 1990, Wheeler & Shaw 1995, Irmiler et al. 1998).

Large-scale industrial peat cutting caused -and still causes- further direct losses of raised bog habitat, as well as indirect losses caused by drainage and subsidence of non-excavated parts of bog massifs (Van der Schaaf 1999, Schouten 2002). Large-scale industrial peat extraction leaves hardly any room for plants and animals during exploitation and in most cases also after abandonment recolonisation of the desiccated bare peat surfaces is problematic (Wheeler & Shaw 1995, Rochefort & Lode 2006). Inlet of alkaline and polluted surface water in bog remnants caused increased nutrient concentrations, decomposition of peat, and disappearance of characteristic vegetation (Lamers et al. 1999) and fauna.

The loss of raised bog habitats and the intensive land use around bog remnants is likely to have caused increased isolation of populations of characteristic species. Intensification of land use around bog remnants has resulted in desiccation (Schouwenaars 1993), whereas agriculture and burning of fossil fuels caused increased atmospheric deposition of nitrogen (N; Berendse et al. 2001) and sulphur (S) compounds (Roelofs 1986, Leuven 1988). Remaining gradients in

bog landscapes structured by groundwater influence may have disappeared as a consequence of lowering of regional groundwater tables. The latter also may have caused increased water table fluctuation in bog remnants, depending on the porosity of remaining peat layers (Vermeer & Joosten 1992, Schouwenaars 1993). Effects of deposition of S compounds and especially N compounds on *Sphagnum* mosses and other bog plants have been intensively studied, showing significant changes in plant species composition and N-content of mosses and vascular plants (e.g., Ferguson & Lee 1980, Aerts et al. 1992, Lamers et al. 2000, Berendse et al. 2001, Bragazza et al. 2005, Limpens et al. 2011). Changes in vegetation structure, plant species composition, and nutritional value of plants have undoubtedly affected the fauna community of raised bogs. A few papers addressed the effects of acidification and eutrophication on bog microbial communities in which testate amoebae are one of the dominant groups (Mitchell et al. 2003, Payne 2010). Contrary to the number of papers on effects of N on *Sphagnum* and other bog plants, papers on the effects of acidification and eutrophication on macroinvertebrates in bogs were lacking.

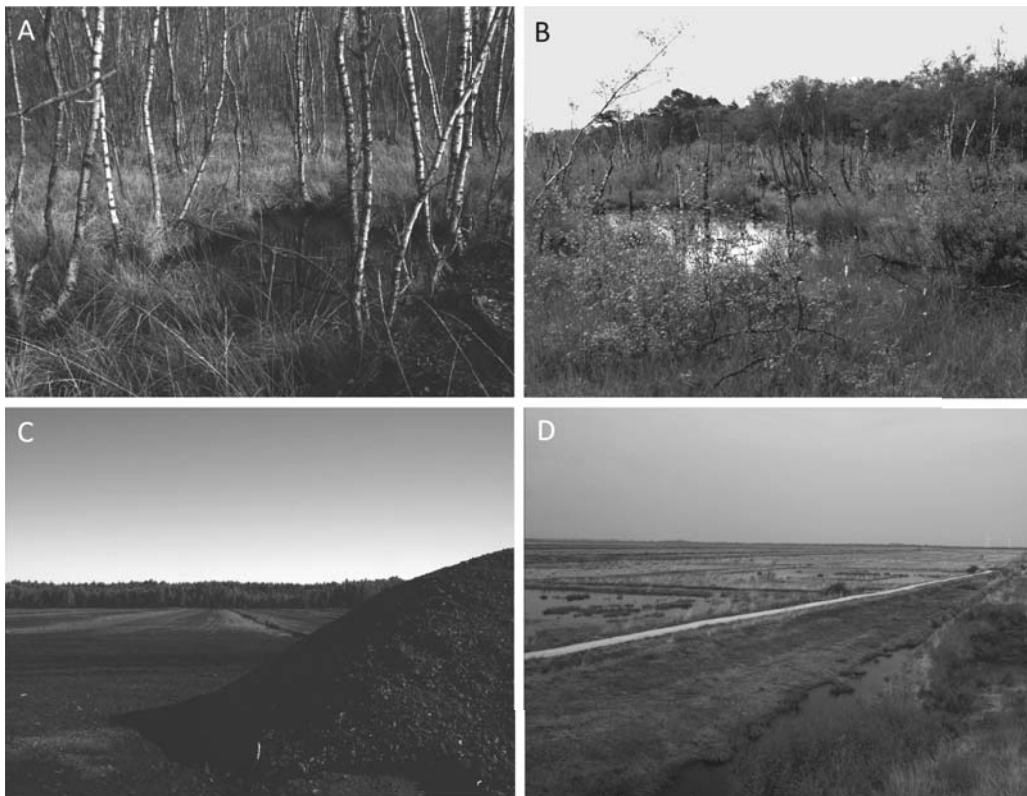


Figure 2. Raised bog remnants differ in baseline conditions prior to implementation of restoration measures, resulting in a wide range of situations after implementation of measures. Small-scale hand peat cutting resulted in mosaics of water filled peat holes and dry strips of peat, overgrown with birch trees (A; Mariapeel, The Netherlands). Situation after raising the water level in such a mosaic (B; Korenburgerveen, The Netherlands). Large-scale industrial peat cutting results in desiccated bare peat substrate (C; Cena, Latvia). Situation after inundation of large-scale industrial peat cutting (D; Stapeler Moor, Germany).

1.4 Conservation and restoration of raised bog landscapes

In the European Union active raised bogs (Habitat type 7110) are listed as priority habitat type in Annex 1 of the Habitats Directive (European Council 1992). Degraded raised bogs still capable of natural regeneration (H7120) and transition mires and quaking bogs (H7140) are also listed as habitat types in Annex 1 of the Habitats Directive. At present the conservation status of these habitat types is bad or inadequate in most European countries (European Environment Agency 2012). This means that intact bogs must be conserved and that bog remnants that are suitable for restoration should be restored. As *Sphagnum* is a keystone genus in raised bogs, restoration measures focus on restoring conditions for the recovery of *Sphagnum*-dominated vegetation, which is a prerequisite for the restoration of functioning raised bog ecosystems (Wheeler & Shaw 1995, Rochefort 2000, Schouten 2002). Consequently, restoration projects are intended to rewet drained bog remnants and include infilling or damming of drainage ditches and the construction of bunds or dykes (Figure 1c) to retain rainwater and decrease fluctuations in the water table (Wheeler & Shaw 1995, Irmeler et al. 1998, Vasander et al. 2003, Blankenburg & Tonniss 2004). Restoration methods including the introduction of *Sphagnum* fragments and seeding or planting of vascular plants have been developed in Europe and North America (Wheeler & Shaw 1995, Sliva & Pfadenhauer 1999, Rochefort & Lode 2006), but have not been widely applied in Europe. Also recurrent management, like grazing or mowing of *Molinia*-dominated vegetation and birch removal is carried out in bog remnants (Irmeler et al. 1998, Tomassen et al. 2003, Natuurmonumenten et al. 2011). Implementation of rewetting and additional measures in sites differing in baseline conditions resulted in a wide range of situations within and between bog remnants: still dry to wet peat substrate and shallowly to deeply inundated peat substrate, in small to large scaled mosaics (Figure 2). Restoration measures were not targeted at restoration of lagg zones or other transitional habitats.

Effective restoration management requires the identification of biotic and abiotic restoration thresholds and adequate measures to overcome these thresholds (Hobbs 2007, Van Kleef 2010). Many investigations concerning raised bog restoration focussed on bog vegetation recovery and key factors involved. The results of rewetting and additional measures vary dependent on the baseline conditions of the bog remnant. Key factors include the type of peat substrate, hydrological conditions, and the proximity of diaspores (Wheeler & Shaw 1995, Rochefort et al. 2003, Tomassen et al. 2003, Smolders et al. 2003, Vasander et al. 2003).

Evaluations of bog restoration measures including fauna diversity have been rare and have usually dealt with only one specific taxonomic group and one specific area (e.g., Utschick 1990, Heaver & Eversham 1991, König 1992, Mossakowski & Främbis 1993, Irmeler et al. 1998). Buttler et al. (1996) showed that the testate amoebae fauna of raised bogs can recover rapidly and fully, regardless of the initial condition of the cutover surface, provided that a *Sphagnum* vegetation is restored. A recent study on flies (Brachycera) in previously mined bogs seven years after implementation of the Canadian restoration approach led to a similar conclusion (Taillefér & Wheeler 2012). Heaver & Eversham (1991) found colonisation of peat extraction sites by several scarce and desirable invertebrate species in less than 20 years, probably as a result of the presence of adjacent refugia. Man-made bog pools, dug as part of a Canadian restoration project on a bare peat field, were colonised by some aquatic invertebrates, including Coleoptera species characteristic of bogs within a few years. However, their abundance was lower than in natural bog pools and Odonata were still almost lacking (Mazerolle et al. 2006). Evaluations of the effectiveness of rewetting measures over longer time periods and in European

bog remnants focussing on the rehabilitation of the aquatic invertebrate community were not performed until the study presented in this thesis. Assessments of possible facilitation of the persistence of characteristic species and invasion of non-characteristic species by the effects of degradation were lacking. This is also the case for assessments of limits to fauna rehabilitation posed by increased nutrient availability and loss of ecotopes present in pristine complete raised bog landscapes.

1.5 Aim of this thesis

The aim of this thesis is to assess the effectiveness of restoration measures and the key factors involved in the degradation and rehabilitation of aquatic invertebrate communities in raised bog landscapes in order to improve restoration management practices.

The following main research questions were formulated:

1. What are the effects of degradation of natural transitions from mineral soil systems to ombrotrophic raised bogs and of nutrient enrichment on aquatic invertebrate communities in raised bog landscapes?
2. What is the effectiveness of rewetting measures in the rehabilitation of aquatic invertebrate communities in raised bog landscapes?
3. Which environmental factors facilitate or limit the rehabilitation of aquatic invertebrate communities in raised bog landscapes?

1.6 Scope and research approach

Assessments of the key factors that facilitate or limit the rehabilitation of the aquatic invertebrate fauna require understanding of the effects of past and current pressures and developments – and the mechanisms involved - that resulted in the degradation of raised bog landscapes. Also an effect assessment of rewetting measures is necessary. To assess the effects of degradation and rewetting measures on the invertebrate communities impact categories and appropriate indicators, as well as references and criteria have to be selected.

Nature conservation and restoration practice focus often on conservation or re-establishment of target species or habitats (Hughes et al. 2011), like the natural habitat types and the species listed in the annexes of the Habitats Directive (European Council 1992) for which Natura 2000 sites are designated in the EU member states. Evaluation can use historical reference conditions or reference systems preserved in intact or less degraded conditions elsewhere. In nature management practice it is unrealistic to aim at assessment of every species or even every aspect of biodiversity, including genetic diversity and population structure (Noss 1990, Simberloff 1997). In addition, management regimes for different species that inhabit the same habitat type can conflict. A complicating factor is that the presence and abundance of species can result from degradation of the ecosystem, rather than a situation appreciated from the perspective of ecosystem rehabilitation. Because raised bogs are known as species-poor ecosystems, as pointed out above, an increase in species richness is not necessarily judged as a positive development.

Ecosystem management, focussing on ecosystem processes, is often seen as a solution to problems of single-species management, assuming that a 'healthy ecosystem' would imply healthy populations of all of its species (Simberloff 1997). As a consequence, ecosystem processes or functions may be seen as the first or even only goal in management. Simberloff (1997) also

points at the problem of poor understanding of the mechanisms of an ecosystem. He proposes a key stone species approach that focuses on understanding of the mechanisms that underlie the function and structure of an ecosystem. Insight in the causal mechanisms between environmental conditions resulting from degradation and restoration measures and the occurrence of species (or in the ‘match’ between landscape and species; Verberk 2008) is necessary to understand the key factors and constraints in the rehabilitation of an ecosystem and its biodiversity. For raised bog ecosystems little knowledge exists on the species composition of aquatic invertebrate communities and under which conditions they occur in bog landscapes, either intact, degraded or under restoration. Consequently, invertebrate species that are indicative of the status of the ecosystem are not defined, although the arrival or increase of species characteristic of raised bogs after rewetting is, of course, positively valued. The single key stone genus known for raised bog ecosystems is *Sphagnum* (Van Breemen 1995, Rochefort 2000) and several plant species are known to increase or decrease with degradation due to drainage and increased deposition of atmospheric N and S compounds (Berendse et al. 2001, Limpens et al. 2003, Tomassen et al. 2004).

To assess the effects of degradation and rewetting measures on the aquatic invertebrate communities the study presented in this thesis compared the environmental conditions and species composition between different states of the bog ecosystem:

- A. Natural water bodies in different parts of pristine raised bog landscapes in Estonia, used as reference in this study;
- B. Water bodies that are remnants of former peat cuttings and trenches used for buckwheat culture, abandoned in the first half of the twentieth century in The Netherlands, and
- C. Water bodies created by rewetting in different baseline situations aimed at the restoration of raised bog habitats in The Netherlands.

Despite the decrease in atmospheric N and S deposition in The Netherlands since 1980 (CBS et al. 2012), deposition is among the highest in western Europe (Jonson et al. 1998, Alcamo et al. 2002) and N deposition is considerably higher than at the study areas in Estonia (*c.f.* Pajuste et al. 2002 and Tomassen et al. 2004).

1.7 Outline of the thesis

Figure 3 gives a schematic representation of the pressures and developments that impact the environmental conditions and mechanisms structuring the invertebrate community of raised bog landscapes. The lower grey box includes the indices of the invertebrate community used in the following chapters of this thesis. References and criteria are derived from the environmental conditions and species composition of pristine raised bog landscapes. Together they are used to evaluate the effects of restoration measures and to assess key factors in the rehabilitation of the invertebrate community.

In chapter 2 the aquatic macroinvertebrate species composition and the occurrence of characteristic and rare species of remnant sites (B) and rewetted sites (C) in Dutch bog remnants is compared to assess effects of rewetting measures. This chapter analyses whether the numbers of characteristic and rare species in water bodies are related to the presence of a characteristic vegetation and to the time that had elapsed since restoration measures were taken. The mobility, mode of dispersal, and life cycle differs between species, and so do their habitat demands. Therefore, chapter 3 compares the species composition of the microinvertebrate groups Rotifera,

Copepoda and Cladocera between remnant sites and rewetted sites (B versus C). Chapters 2 and 3, along with chapter 4, shed light on the persistence of species during the processes of degradation and restoration and on the recolonisation of species.

The effects of degradation on aquatic invertebrate communities are investigated in chapters 4 and 5. In chapter 4 it is assessed which species occur in pristine ombrotrophic bog pools and in the groundwater influenced parts of a pristine raised bog landscape (lagg and transitional mire) in Estonia (A). Subsequently, the occurrence of these species in either (C) or not (B) rewetted parts of Dutch bog remnants was investigated (A versus B and C). This comparison is described in chapter 4 for various groups of aquatic macroinvertebrates. Chapter 5 focuses on the oligochaetes to gain further insight in how loss of groundwater influence and increased nutrient availability has affected the species occurrence in The Netherlands. In addition, chapter 4 addresses the effectiveness of restoration by assessing which species profit from the restoration measures taken in Dutch bog remnants.

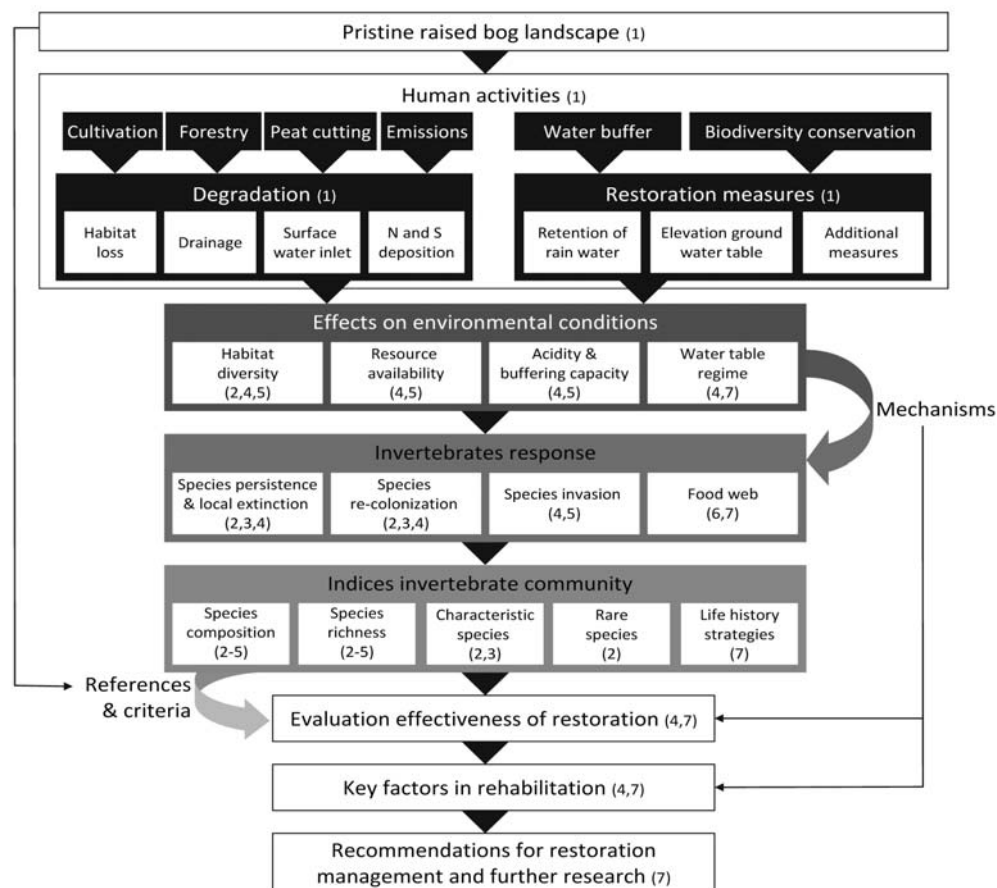


Figure 3. Schematic representation of the past and current human activities that impact the environmental conditions and mechanisms structuring the invertebrate community of raised bog landscapes. The lower half of the scheme represents the indices, references and criteria used in the evaluation of restoration measures and assessment of key factors in the rehabilitation of the invertebrate community. The numbers of the chapters dealing with the respective aspects are presented between brackets.

Chapter 1

The increased nutrient availability may impact the role of various food sources in the invertebrate food web in raised bog pools. Therefore, chapter 6 investigates the importance of different potential food sources in the invertebrate food web of pristine bog pools in Estonia, using stable isotopes and phospholipid-derived fatty acids (PLFAs).

Chapter 7 presents a synthesis of the findings described in the chapters 1-6 and related studies. The effectiveness of restoration measures and constraints in the rehabilitation of aquatic invertebrate communities in raised bog landscapes are discussed and recommendations for the restoration practice are given to help nature managers to improve the effectiveness of their practices. Finally, recommendations for further research on fundamental and applied issues concerning the functioning of raised bog ecosystems are outlined.

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Chapter 2

Do restoration measures rehabilitate fauna diversity in raised bogs? A comparative study on aquatic macroinvertebrates.

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◀ Aquatic invertebrate species characteristic for raised bog landscapes have survived the process of bog degradation in hand peat cutting pits like this one in the bog remnant Mariapeel, The Netherlands.

Abstract

To assess whether raised bog restoration measures contribute to the conservation and restoration of the fauna diversity, macroinvertebrate species assemblages were compared between water bodies created by rewetting measures and water bodies which have not been subject to restoration measures, but are remnants of former peat cuttings and trenches used for buckwheat culture in the past.

The restoration sites were inhabited by characteristic raised bog species and rare species, but their numbers were higher at the remnant sites not affected by restoration management. A considerable number of characteristic and rare fauna species were only found at the remnant sites. The remnant sites included considerably more variation in macroinvertebrate species assemblages and had a higher cumulative species richness.

The number of characteristic macroinvertebrate species was not clearly related to the presence of a characteristic raised bog vegetation. In restoration sites numbers of rare and characteristic species per site tended to increase with the time elapsed after rewetting. However, restoration measures will not automatically result in restoration of a more or less complete macroinvertebrate species spectrum, as restoration measures have so far resulted in habitats for only a limited number of the characteristic species.

When planning restoration measures, it is recommended to protect the populations of rare and characteristic species present in the area, as these populations may become the sources for colonisation of rewetted sites. Safeguarding habitat diversity during the restoration process and restoration of different elements of the habitat diversity of complete raised bog systems will result in the characteristic fauna diversity being conserved and restored more successfully.

2.1 Introduction

At the moment rewetting measures are taken in many raised bog remnants in western Europe to conserve and restore raised bog flora and fauna. Small- and large-scale drainage, peat cutting and cultivation (e.g. buckwheat culture) have resulted in the degradation and disappearance of raised bog systems (Verhoeven 1992, Schouten et al. 1998). It can be expected that a number of characteristic fauna species have declined or disappeared as a consequence of the disappearance of the various habitats of pristine raised bog systems. At the same time, however, human activities have also resulted in new habitats within the bog landscape. Dehydration and mineralisation of peat and the inlet of minerotrophic or nutrient-rich water have changed water and soil qualities (Lamers et al. 1998a and 1998b). As a result, species which do not occur under ombrotrophic (i.e., acid, nutrient-poor) conditions were able to establish in bog remnants (Göttlich 1980, Wheeler & Shaw 1995, Irmeler et al. 1998). For some of these species, the original habitats have been degraded or have disappeared as a result of cultivation, making bog areas nowadays a refugium for species which were originally not dependent on raised bogs (Akkermann 1982, Schouwenaars et al. 2002). During the last century, flora and fauna species assemblages in raised bog areas have also been affected by increased nutrient availability due to increased nitrogen deposition (Bobbink et al. 1998).

During the last four decades drainage ditches have been blocked and bunds and weirs have been build to rewet drained and cutover raised bog remnants with rainwater. In some cases, these

measures have resulted in the recovery of a *Sphagnum* vegetation or the formation of floating rafts on which a characteristic hummock-hollow vegetation establishes, including e.g. *Sphagnum magellanicum*, *S. papillosum*, and *S. rubellum* (Verhoeven 1992, Lamers et al. 1999). However, *Betula* sp. and *Molinia caerulea* have invaded most of these sites and large areas of inundated cutover peat still consist of open water with only *Sphagnum cuspidatum* (Schouten et al. 1998).

The aims of nature management generally include the conservation and restoration of a complete species spectrum, paying special attention to characteristic and threatened or Red-Listed species (Bal et al. 2001). The success of raised bog restoration measures is mainly evaluated on the basis of the development of a *Sphagnum*-dominated vegetation, as well as the presence of characteristic *Sphagnum* and vascular plant species. Evaluations of restoration measures including fauna diversity have, however, been rare and have usually dealt with only one specific taxonomic group and one specific area (e.g. Utschick 1990, König 1992, Mossakowski & Främbis 1993, Irmeler et al. 1998). Buttler et al. (1996) showed that the testate amoebae fauna of raised bogs can recover rapidly and fully, regardless of the initial condition of the cutover surface. Since most restoration projects do not include a monitoring programme, it is generally unknown whether they have had any effects on the fauna, whether positive or negative. Also, little attention is being paid to fauna diversity in the planning of management measures.

To assess the effects of restoration measures on fauna and identify the key factors in successful conservation and restoration of fauna, a study of aquatic macroinvertebrates in Dutch raised bog areas was started. Aquatic macroinvertebrates were chosen, because most measures in raised bog restoration are focussed on water quantity and quality. The present study compared species assemblages between (1) water bodies created by rewetting measures aimed at the restoration of raised bog habitats and (2) water bodies which have not been subject to restoration measures, but are remnants of former peat cuttings and trenches used for buckwheat culture, abandoned in the first half of the twentieth century. These two groups of water bodies will be referred to as restoration sites and remnant sites, respectively. Conservation and restoration in raised bog areas should include not only the characteristic bog species, but also those species which are nowadays more or less dependent on raised bog areas for their survival in the Netherlands. Therefore, the present paper does not focus only on species characteristic of pristine bogs, but also takes into account the present distribution of non-characteristic macroinvertebrate species.

The present paper addresses the question whether remnant and restoration sites differ in species assemblage, species richness, and the numbers of characteristic and rare fauna species. A second question was whether the numbers of characteristic and rare species in these water bodies could be related to the presence of a characteristic vegetation and to the time that had elapsed since restoration measures were taken.

2.2 Materials and methods

Sampling sites

Aquatic macroinvertebrates were sampled at 47 sites in 7 raised bog areas (Figure 1). Sampling sites were chosen to include most of the various types of water body present. The water bodies sampled differed in age, size, water and substrate quality, vegetation composition and structure. Twenty-seven of the water bodies sampled were created by rewetting measures, 20 were remnants of former peat cutting or trenches used in buckwheat culture, which had been in existence for

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more than 50 years and had not been subject to bog restoration measures. These sites were in different stages of secondary succession.



Figure 1. Locations of raised bog areas in the Netherlands at which samples were taken. 1. Fochteloërveen (0/4), 2. Dwingelerveld (8/0), 3. Bargerveen (1/10), 4. Haaksbergerveen (1/9), 5. Korenburgerveen (4/0), 6. Mariapeel (0/4), 7. Tuspeel (6/0). Figures in brackets are the numbers of remnant and restoration sites sampled at each of the areas.

Sampling method

Macroinvertebrates were sampled using a 20x30 cm pond net with $\frac{1}{2}$ mm mesh size. Most samples consisted of a 1 m sweep starting from the substrate and more or less open water into more dense vegetation near the shore. If the water body only included open water, one or more longer sweeps were taken to collect at least 100 macroinvertebrate individuals in a sample. In very dense *Sphagnum* vegetation, 20 to 50 cm sweeps were made to avoid the pond net becoming clogged with *Sphagnum*. As fauna abundance is generally high in this dense vegetation, most of these small samples contained over 100 individuals, or even more than 1000. Only in two small remnant water bodies were fewer than 100 individuals caught.

Each site was sampled both in spring 1999 and autumn 1998 or 1999, except four temporary remnant water bodies, which could only be sampled in either spring or autumn. Samples were transported to the laboratory and stored until analysis at 4°C. The collected material was washed over three sieves with 2, 1, and $\frac{1}{2}$ mm mesh sizes respectively and sorted in white trays. All macroinvertebrates were identified to species level if possible, except Coleoptera larvae and Oligochaetes, which were excluded from the data analysis.

Environmental variables

The year in which the restoration sites had been rewetted was derived from management reports or provided by the local manager. Variables assessed in summer for all sampling sites included

the presence of open water, *Sphagnum*, floating leaves of higher plant species, trees and organic sediment, as well as the mean *Sphagnum* density in numbers of capituli per dm², and whether the water body was permanent or temporary. In spring, summer and autumn field assessments included the depth of the water body, surface area, electric conductivity and pH and samples of the surface water were taken. Samples were stored overnight at 4°C, and turbidity, pH and alkalinity were measured the next day. After 1 mg citric acid per 5 ml of water had been added, samples were stored at -20°C until analysis. The following were determined colorimetrically; NO₃⁻ according to Grasshoff & Johannsen (1972), NH₄⁺ according to Kamphake et al. (1967), PO₄³⁻ according to Henriksen (1965). Cl⁻ according to O'Brien (1962). Na and K were determined with a Technicon flame photometer. Ca, Mg, Fe, Al, P and S were measured by inductively coupled plasma emission spectrometry (Jarell Ash Plasma 200, Instrumentation Laboratory). Total inorganic carbon in surface water was measured once with an 'Oceanography International' model 0525 HR infrared carbon analyser on autumn samples. Oxygen content was determined once, using the Winkler titration (Drew & Robertson 1974, Carpenter 1965) on samples collected in the autumn during daytime. For those environmental variables that were measured more than once, average values were used in the data analyses.

Classification of macroinvertebrate species

Macroinvertebrate species were considered to be characteristic of raised bogs if they were listed in literature as acidophilous, acidobiontic, tyrphophilous, tyrphobiontic or typical of raised bogs. These data and data on species rareness in the Netherlands were taken from Peus (1923), Nieser (1982), Geijskes & Van Tol (1983), Drost et al. (1992), Higler (1995), Duursema (1996), Bos & Wasscher (1997), Wasscher et al. (1998), Smit & Van der Hammen (2000), Nijboer & Verdonchot (2001) and from information provided by an expert (Dr H.K.M. Moller Pillot, pers. comm.). Red Lists were available for Turbellaria, Odonata, Ephemeroptera and Trichoptera (Wasscher et al. 1998, Verdonchot et al. 2003). For Coleoptera, the provisional Red List presented by Drost et al. (1992) was used.

Vegetation and macroinvertebrates indices

The species composition of the vegetation in each water body and the adjacent vegetation was assessed by estimating plant species cover, using cover class numbers 1 to 7 for the classes sporadic, rare, occasional, frequent, abundant, co-dominant and dominant from the scale proposed by Tansley (1946). Each plant species was assigned a score based on rareness, trend and desirability from the point of view of raised bog restoration (Table 1). Ten points were assigned for the hummock-building *Sphagnum* species *S. magellanicum*, *S. papillosum*, and *S. rubellum* and for a permanent floating raft with *Sphagnum* species. Five points were assigned for species which are rare and declining in the Netherlands (Van der Meijden, 1996) and are characteristic of raised bog vegetation. Undesirable species like *Betula* sp. and *Molinia caerulea* were assigned no points. Relatively common, but not undesirable species were assigned 2.5 points. A Vegetation Quality Score (VQS) was calculated for each sampling site by the sum of the species cover class multiplied by the species score and dividing this by the sum of all species cover class numbers.

$$VQS = \frac{\sum_{i=1}^n (\text{score of species}_i * \text{cover class}_i)}{\sum_{i=1}^n \text{cover class}_i}$$

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Table 1. Plant species found at sampling sites and scores assigned to them.

Plant species	Score
<i>Sphagnum magellanicum</i> , <i>S. papillosum</i> , <i>S. rubellum</i> , permanent floating raft with <i>Sphagnum</i> sp.	10
<i>Calluna vulgaris</i> , <i>Erica tetralix</i> , <i>Eriophorum angustifolium</i> , <i>Eriophorum vaginatum</i> , <i>Menyanthes trifoliata</i> , <i>Rhynchospora alba</i> , <i>Sphagnum denticulatum</i> , <i>Utricularia minor</i>	5
<i>Agrostis canina</i> , <i>Carex rostrata</i> , <i>Hydrocotyle vulgaris</i> , <i>Potamogeton natans</i> , <i>Potentilla palustris</i> , <i>Sphagnum cuspidatum</i>	2.5
<i>Azolla filiculoides</i> , <i>Betula</i> sp., filamentous green algae, <i>Juncus effusus</i> , <i>Molinia caerulea</i> , <i>Phragmites australis</i> , <i>Quercus robur</i> , <i>Salix</i> sp.	0

Based on Foster (1990), a Fauna Species Quality Score (FSQS) was calculated for each sampling site. A similar procedure has also been applied by e.g. Painter (1999) and Oertli et al. (2002). All species were assigned a species score, depending on their rareness class in the Netherlands. The species scores of very common, common, fairly common, fairly rare, rare and very rare species were 1, 2, 4, 8, 16 and 32, respectively. For characteristic species, the species score was multiplied by two. The FSQS of a site was the average score of all species present.

$$FSQS = \frac{\sum_{i=1}^n \text{score of species}_i}{\text{total number of species}}$$

Data analysis

Data on the presence of macroinvertebrate species in the spring and autumn samples were pooled for each of the 47 sites. Correspondence Analysis (CA) of these data were performed in Canoco for Windows version 4.0 (Ter Braak & Smilauer 1998). The significance of each environmental variable was tested with the Monte Carlo resampling procedure (500 permutations). Cumulative species richness curves were based on averages of 50 random sorts of the sampling sites using BioDiversityProfessional Beta 1 (McAleece 1997).

To determine whether correlations existed between VQS or site age and the numbers of rare and characteristic macroinvertebrate species or FSQS, Pearson correlation coefficients and their significance were calculated using SPSS. Significance of differences in the numbers of rare and characteristic macroinvertebrate species, FSQS and VQS between remnant and restoration sites was tested using the Student T-test.

2.3 Results

Fauna species assemblages

The correspondence analysis plot of the sampling sites is shown in Figure 2. The site scores were calculated from the macroinvertebrate species presence data only. Remnant sites and restoration sites were clearly separated on the first and second CA-axis, which explained 7.4% and 6.4% of the variation in species data, respectively. Restoration sites were plotted very close together compared to remnant sites. This indicates that the degree of variation in species assemblages was relatively low at the restoration sites.

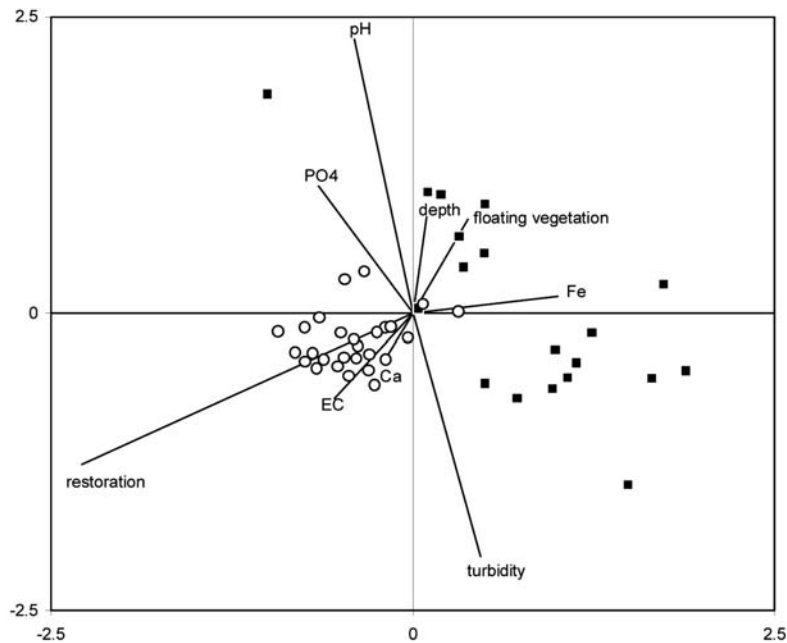


Figure 2. Correspondence Analysis plot of sampling sites based on the presence of macroinvertebrate species. Significant environmental variables are shown as lines. Open circles represent restoration sites and filled squares represent remnant sites.

When the variable restoration vs. remnant site was excluded, 66.0% of the total variance in species data could be explained by all other environmental variables. Adding this variable resulted in an increase of total explained variation of 2.5%. Restoration vs. remnant alone explained 6.0% of the total species variation. This is equal to 8.8% of the variance explained by all environmental variables and to 81% of the variation explained by the first CA-axis. After the deletion of alkalinity, total P and Na – because of high correlations with pH, PO_4 and Cl respectively – the variables restoration vs. remnant site, pH, electric conductivity, Ca, Fe, PO_4 , depth, presence of floating leaves and turbidity contributed significantly ($P < 0.05$) to explaining the variation in fauna data. These significant variables together accounted for 34.5% of the variation in fauna data. These variables were plotted inert in the CA-plot in Figure 2.

Species richness

At the 47 sampling sites a total of 149 macroinvertebrate species sampled were found (Table 2). Of these, 133 species were found at the 20 remnant sites, versus 100 species at the 27 restoration sites. Eighty-four species were found at both remnant and restoration sites. Figure 3 shows cumulative species richness curves for remnant sites and restoration sites. Apart from the difference in total species richness, the difference in the shape of the curves is remarkable. Both curves are still rising at their ends, but the slope of the remnant sites curve is steeper than that of the restoration sites curve. Species richness per site did not differ significantly between the remnant and restoration sites. However, the number of individuals collected per site was significantly lower at the remnant sites (Table 3).

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Table 2. Numbers (N) and relative numbers of macroinvertebrate species found at all sampling sites together, remnant sites and restoration sites, and numbers of species found at both remnant sites and restoration sites, for different classes of rareness in the Netherlands. n= number of sampling sites.

Rareness class	All sites n=47		Remnant sites n=20		Restoration sites n=27		Species shared
	N	%	N	%	N	%	N
Very common	37	24.8	33	24.8	28	28.0	24
Common	37	24.8	33	24.8	26	26.0	22
Fairly common	34	22.8	31	23.3	23	23.0	20
Fairly rare	29	19.5	24	18.0	18	18.0	13
Rare	8	5.4	8	6.0	5	5.0	5
Very rare	4	2.7	4	3.0	0	0.0	0
Total species number	149	100.0	133	100.0	100	100.0	84

Table 3. Averages ($\pm$ sd) of the total numbers of individuals, species richness, numbers of characteristic and more or less rare characteristic species (CR in Table 4), Fauna Species Quality Scores (FSQS) and Vegetation Quality Scores (VQS) of the sampling sites. Significance of the differences between remnant and restoration sites is indicated as the P-values in the T-test. P-values < 0.05 are in italics. n= number of sampling sites.

	Remnant sites n=20	Restoration sites n=27	T-test P
Individuals/sampling site	576 $\pm$ 388	1276 $\pm$ 932	<i>0.001</i>
Species richness	24.0 $\pm$ 15.4	22.7 $\pm$ 5.8	0.722
Characteristic species	6.7 $\pm$ 4.3	5.1 $\pm$ 3.3	0.153
Rare characteristic species	3.6 $\pm$ 2.5	2.4 $\pm$ 2.1	0.063
FSQS	6.4 $\pm$ 2.0	4.5 $\pm$ 1.8	<i>0.001</i>
VQS	3.6 $\pm$ 1.8	2.5 $\pm$ 1.8	<i>0.041</i>

Species rareness, characteristic species, quality scores, and Red-Listed species

Thirty-six of the 41 fairly rare, rare, and very rare species were sampled at the remnant sites (Table 2), while the restoration sites yielded only 23 more or less rare species. All 12 rare and very rare species found were present at at least one of the remnant sites, whereas 5 of them were found at restoration sites. Species more or less common in the Netherlands represented 72.9% (97 species) of the total number of species at the remnant sites and 77.0% (77 species) at the restoration sites.

Species characteristic of raised bog systems or included in the Red List are listed in Table 4. Thirty-six of the 149 species collected are more or less characteristic of water bodies in raised bog systems. Thirty-four of them were found at the remnant sites and 24 at the restoration sites. Of the 11 fairly common characteristic species, only 1 species was not found at the restoration sites, while 7 of the 19 fairly rare characteristic species were not found. Two of the 4 rare and none of the 2 very rare characteristic species were found at the restoration sites.

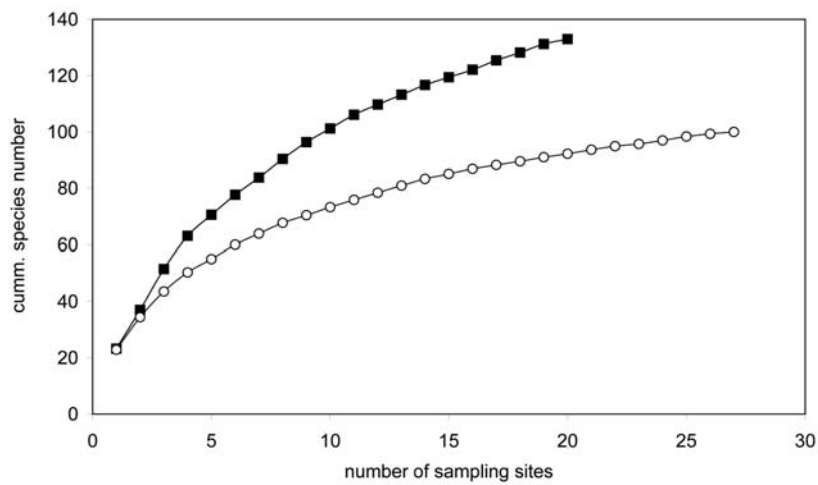


Figure 3. Cumulative macroinvertebrate species richness curves of 27 restoration sites and 20 remnant sites composed of averages of 50 random sorts of sampling sites. Open circles for restoration sites and filled squares for remnant sites.

Some of the characteristic species were frequently found, most of these being fairly common in the Netherlands (Table 4). The average numbers of characteristic species and of fairly rare, rare, and very rare characteristic species (CR in Table 4) sampled per site were higher at the remnant sites, although the difference was not statistically significant (Table 3). The Fauna Species Quality Score and the Vegetation Quality Score were significantly higher at the remnant sites.

Of the 149 species collected, 11 are on the Red List. Seven of them are characteristic of raised bogs. The 4 non-characteristic Red-Listed species were only found at 3 different remnant sites. Both at remnant sites and restoration sites 5 characteristic Red-Listed species were found.

Correlations with VQS and site age

For the 20 remnant sites, the number of characteristic species per site was significantly correlated with VQS ($r=0.49$, $P=0.030$; Figure 4). The number of more or less rare characteristic species (CR in Table 4) and FSQS were not significantly correlated with VQS ($r=0.28$, $P=0.235$ and $r=-0.09$, $P=0.709$, respectively). For the 27 restoration sites, only the FSQS was correlated with the VQS ($r=0.60$, $P=0.001$), while no significant correlation was found between VQS and the number of characteristic and rare characteristic species ($r=0.20$, $P=0.329$ and $r=0.373$, $P=0.055$, respectively).

No significant correlation was found between site age and the number of characteristic or rare characteristic species at the restoration sites ($r=0.25$, $P=0.214$ and $r=0.33$, $P=0.092$, respectively), although a positive trend was found (Figure 5). However, FSQS and the number of fairly rare and rare species per site were correlated with the number of years elapsed after rewetting measures were taken ($r=0.50$, $P=0.009$ and $r=0.48$, $P=0.012$, respectively).

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Table 4. Numbers of sampling sites at which characteristic species (C), more or less rare characteristic species (CR), and Red-Listed species (RL) were found. The taxonomic group to which species belong is abbreviated: Hydrachnidia (hyd), Chaoboridae (cha), Chironomidae (chi), Coleoptera (col), Cylindrotomidae (cyl), Heteroptera (het), Odonata (odo), and Trichoptera (tri). Red List (RL) classes are: strongly threatened (1), threatened (2), and vulnerable (3). Rareness classes are fairly common (fc), fairly rare (fr), rare (r), and very rare (vr). Tyrphobiontic (tb), tyrphophilous (tp), acidobiontic (ab) and acidophilous (ap). n= number of sampling sites.

Species name	Taxonomic group	C	CR	Rareness class	RL class	tb	tp	ab	ap	All sites n=47 numbers of sampling sites	Remnant n=20	Restoration n=27
<i>Ilybius aenescens</i>	col	x	x	fr		x				11	2	9
<i>Acilius canaliculatus</i>	col	x	x	fr			x			13	6	7
<i>Agabus congener</i>	col	x	x	r			x			3	3	0
<i>Berosus luridus</i>	col	x	x	fr			x			1	0	1
<i>Bidessus spec.</i>	col	x	x	fr	1	x				4	3	1
<i>Enochrus affinis</i>	col	x		fc		x				13	9	4
<i>Helophorus flavipes</i>	col	x	x	r		x				2	2	0
<i>Hydroporus gyllenhalii</i>	col	x	x	fr				x		5	5	0
<i>Hydroporus melanarius</i>	col	x	x	fr				x		3	3	0
<i>Hydroporus obscurus</i>	col	x	x	fr				x		11	7	4
<i>Hydroporus pubescens</i>	col	x		fc				x		8	6	2
<i>Hydroporus tristis</i>	col	x		fc				x		18	14	4
<i>Arrenurus stecki</i>	hyd	x	x	fr					x	18	5	13
<i>Hydroporus scalesianus</i>	col	x	x	fr	2				x	4	2	2
<i>Nartus grapii</i>	col	x	x	fr	2				x	1	1	0
<i>Oxus nodigerus</i>	hyd	x	x	r					x	5	4	1
<i>Agrypnia obsoleta</i>	tri	x	x	fr	3					3	0	3
<i>Arrenurus neumani</i>	hyd	x	x	fr						1	1	0
<i>Ceriagrion tenellum</i>	odo	x	x	fr						8	4	4
<i>Coenagrion lunulatum</i>	odo	x		fc	1					14	1	13
<i>Cymatia bondsdorffi</i>	het	x		fc						14	1	13
<i>Enochrus ochropterus</i>	col	x	x	fr						11	5	6
<i>Gerris gibbifer</i>	het	x	x	fr						1	1	0
<i>Graphoderus zonatus</i>	col	x	x	fr						1	1	0
<i>Hebrus ruficeps</i>	het	x		fc						9	5	4
<i>Hesperocorixa castanea</i>	het	x	x	fr						4	4	0
<i>Hydroporus umbrosus</i>	col	x		fc						16	7	9
<i>Laccophilus ponticus</i>	col	x	x	vr	1					1	1	0
<i>Lasiodiamesa spec.</i>	chi	x	x	vr						4	4	0
<i>Leucorrhinia dubia</i>	odo	x		fc	3					4	2	2
<i>Leucorrhinia rubicunda</i>	odo	x		fc						18	5	13
<i>Macropelopia adaucta</i>	chi	x		fc						4	4	0
<i>Micropsectra fusca</i>	chi	x	x	fr						3	2	1
<i>Mochlonyx fuliginosus</i>	cha	x	x	r						8	6	2
<i>Phalacrocerca replicata</i>	cyl	x	x	fr						24	6	18
<i>Psectrocladius platypus</i>	chi	x		fc						16	6	10
<i>Cybister lateralimarginalis</i>	col			fc	2					1	1	0
<i>Hagenella clathrata</i>	tri			vr	3					1	1	0
<i>Limnephilus marmoratus</i>	tri			fr	3					1	1	0
<i>Limnephilus nigriceps</i>	tri			vr	2					1	1	0
Total numbers of species												
Remnant sites		34	23		10	1	5	5	4	38	38	
Restoration sites		24	14		5	1	4	3	3	24		24
All sites		36	25		11	1	6	5	4	40		

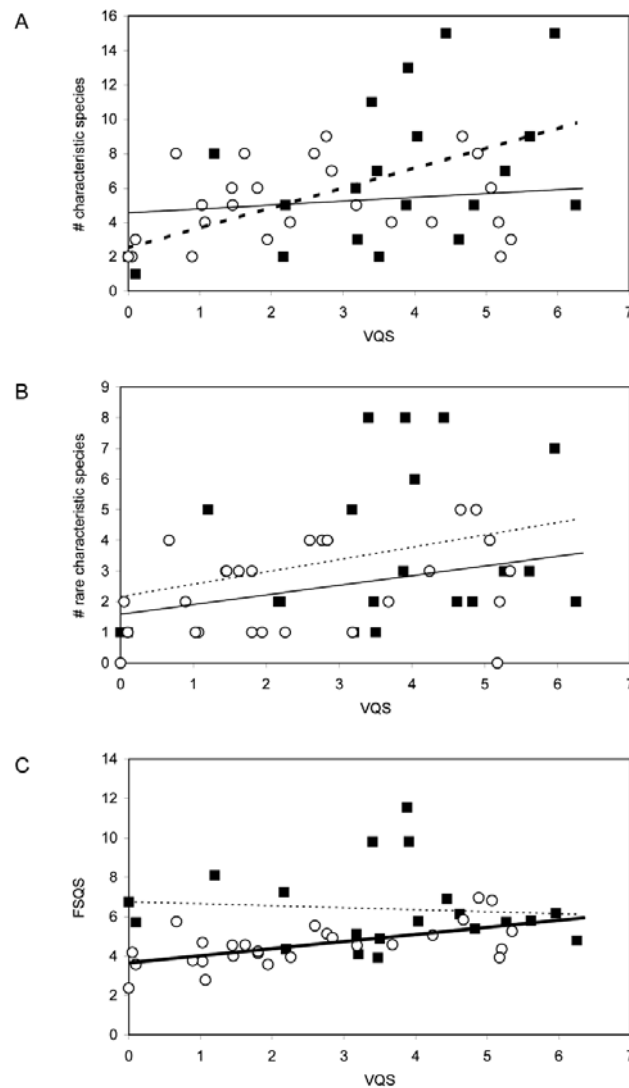


Figure 4. Relation between the numbers of a) characteristic species and b) more or less rare characteristic species (CR in Table 4) and c) Fauna Species Quality Scores (FSQS), and the Vegetation Quality Scores (VQS) for 20 remnant sites and 27 restoration sites. Significant correlations are indicated by bold trend lines, the others by ordinary lines. Uninterrupted trend lines and open circles are used for restoration sites, dotted lines and filled squares for remnant sites. For explanation of statistics see text.

2.4 Discussion

Macroinvertebrate species assemblages appear to differ between restoration sites and remnant sites. Both restoration sites and remnant sites were inhabited by a number of characteristic, rare and Red Listed macroinvertebrate species. However, larger numbers of characteristic species and rare species were found at the remnant sites, resulting in the higher Fauna Species Quality Score (FSQS) at the remnant sites. The cumulative species richness was also high compared to the restoration sites and more than a quarter of the characteristic species was only found at the

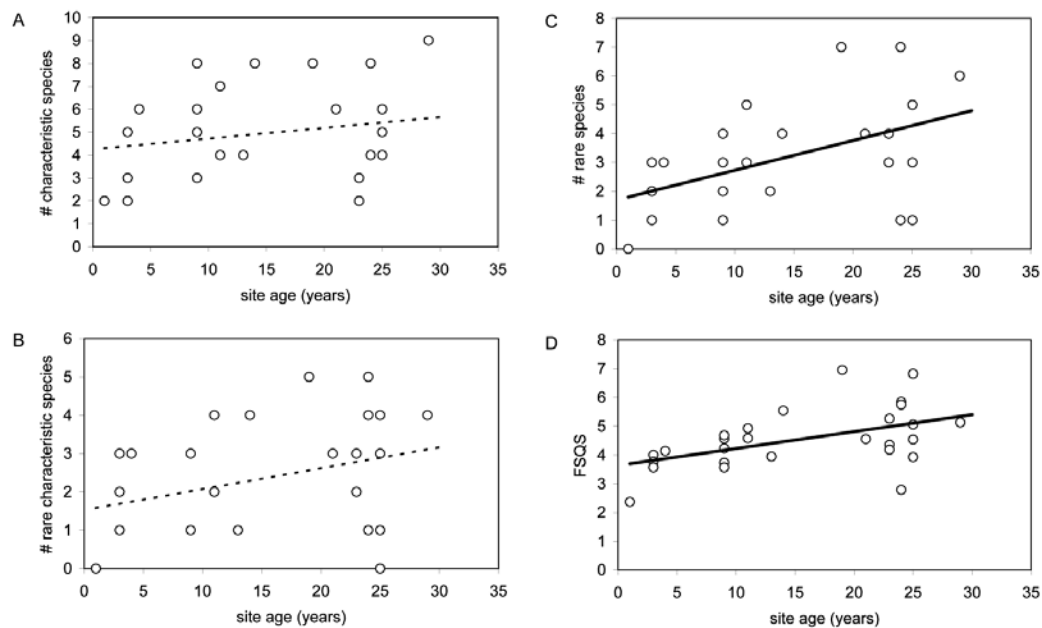


Figure 5. Numbers of a) characteristic species, b) more or less rare characteristic species (CR in Table 4) and c) more or less rare species and d) Fauna Species Quality Scores (FSQS) for the 27 restoration sites, plotted against site age. Significant correlations are indicated by uninterrupted trend lines, the others by dotted trend lines. For explanation of statistics see text.

remnant sites. This difference is strengthened by the fact that the numbers of individuals collected were significantly lower at the remnant sites. What could be the causes of these differences between remnant sites and restoration sites?

Important factors structuring aquatic macroinvertebrate species assemblages of inland waters in the Netherlands are salinity, current velocity, dimensions of water bodies, duration of drought periods, acidity and trophic state (Leuven et al. 1987a and 1987b, Van der Hammen 1992, Verdonschot et al. 1992). Some of these, or related, variables significantly explained the variation in the species assemblages within the group of remnant sites as well as within the group of restoration sites, but not clearly between these two groups of sites. Besides these factors related to habitat quality, site age and colonisation time may be important, as Painter (1999) and Fairchild et al. (2000) showed for aquatic beetles. Site age was not analysed in our correspondence analysis, as the age of most of the remnant sites was unknown. However, it is important to know whether age is an important causal factor and what restoration measures will contribute in due time to the conservation and restoration of a complete species spectrum within the Netherlands.

The FSQS of restoration sites was found to increase with site age. This was mainly due to the correlation between the numbers of fairly rare and rare species and the site age. The numbers of characteristic and rare characteristic species were not significantly correlated with the site age. The age of the oldest restoration site sampled in the present study was 29 years. The restoration sites might be expected to become recolonised by larger numbers of characteristic and rare

species after a longer time period, on the conditions that (1) adjacent populations of these species are present and (2) the sites meet the habitat requirements of the species. The presence of adjacent populations is important in the highly fragmented landscape of the Netherlands (Den Boer 1990, De Vries 1996), especially for raised bog species and a substantial number of rare species, as raised bog areas have become rare and a considerable number of species are hardly able to disperse over long distances. Biggs et al. (2001) found that the restoration of the river Cole (UK) was successful because its upper reaches and stagnant water bodies along the river harboured many species, which were able to recolonise the river after restoration. This stresses the importance of remnant sites as refugia until restoration sites have been recolonised by the species in question.

Regarding the habitat requirements of the various macroinvertebrate species, further analysis of the ecological traits of the macroinvertebrates are necessary to understand differences in species assemblages within and between remnant and restoration sites. The present data do not allow the conclusion that restoration measures will after some time result in suitable habitats for the species diversity, especially for the characteristic species, present at the remnant sites. The cumulative species richness of remnant sites was relatively high, whereas species richness per site did not differ between the remnant and restoration sites. This means that the remnant sites included much more variation in species assemblages, which is also indicated by the different ranges of site scores in the CA-plot. Extrapolation of the cumulative species richness curve indicates that more restoration sites will not result in a major increase in species numbers. So far, rewetting measures in various initial situations have resulted in a habitat for a limited number of fauna species, including only some of the characteristic raised bog species. Currently, a considerable number of characteristic and rare species are still dependent on remnant sites for their survival in raised bog areas, and in the Netherlands as a whole.

The number of characteristic macroinvertebrate species was not clearly related to the presence of a characteristic raised bog vegetation. Sites with a successful restoration of a characteristic raised bog vegetation (high VQS) do not necessarily have larger numbers of characteristic macroinvertebrate species. The VQS of the remnant sites with the largest numbers of more or less rare characteristic fauna species was moderate. The dominant plant species at these sites were *Betula* sp., *Molinia caerulea* and *Sphagnum cuspidatum*, with some *Eriophorum angustifolium*. These sites are actually the only known find-spots in Western Europe of the very rare and characteristic chironomid species *Lasiodiamesa gracilis* (cf. Brundin 1966).

What do these results mean for restoration management in raised bog areas? Rewetting is necessary to restore hydrological and biogeochemical processes of raised bog systems in order to restore a characteristic hummock-hollow vegetation. If a restoration site initially consisted of cutover bare peat, the establishment of some characteristic plant and fauna species is a nice result of the restoration measures taken. However, it is worse when the area initially included remnant sites with some characteristic species, which are now substituted by a species assemblage like all other restoration sites, excluding previously present characteristic and rare species. Therefore, raised bog restoration should not focus everywhere on creating suitable conditions only for the hummock-hollow vegetation type.

Studies on several groups of aquatic macroinvertebrates in Dutch raised bog areas (Verberk et al. 2001) and in relatively pristine raised bog systems in Ireland and Estonia (De Leeuw 1986, Smits et al. 2002) show that characteristic species do not all occur in ombrotrophic raised bog

centres. Although several characteristic species might only depend on e.g. large, open pools or shallow pools with dense *Sphagnum* vegetation in bog centres, other species need other elements of the raised bog system, like transitional habitats or water bodies with some water flow or the influence of minerotrophic water. In addition, several species depend on environmental conditions which only occur in gradients or need a combination of different habitats to complete the different stages in their life cycles (Verberk et al. 2001, Schouwenaars et al. 2002), or for longer-term survival in case of unfavourable periods in one or more of the present habitat types (Settele et al. 1996, pers. comm. Dr H.K.M. Moller Pillot). Habitat diversity within an area can offer more suitable habitats, resulting in greater species richness (Harper et al. 1997). Thus, conservation and restoration of habitat diversity is necessary to meet the requirements of the species diversity of complete raised bog systems.

In conclusion, efforts to improve the success of restoration measures in raised bog areas need to focus first on the conservation of the present refugia of characteristic bog species or species that have become rare. Therefore, it is necessary to assess which species are present in the various habitat types, before any measures are taken. Such assessments should not neglect sites which do not seem very valuable on the basis of their vegetation composition. Next to the assessment of the present fauna species, it is important to know the habitat conditions that species depend on and the regional and local hydrological and biogeochemical processes causing these conditions. Just these processes are influenced by such restoration measures as building bunds and weirs to manipulate e.g. water table fluctuations and water flow patterns. This knowledge is essential in choosing the restoration strategy which will conserve populations in the short term and will restore characteristic flora and fauna species assemblages in the various parts of the raised bog system in the longer term (Schouten et al. 1998). In most cases, measures have to be phased in time and space, but the precise time scale to be used depends on the distribution and habitat requirements of the species concerned and on the progress of restoration and recolonisation, as well as on the possible side-effects of measures. When monitoring has shown that the species concerned have colonised other parts of the area, (phased) measures can be taken to restore conditions for other target species to recover. Safeguarding the necessary habitat diversity during the restoration process and restoring different elements of the habitat diversity of complete raised bog systems will result in the characteristic fauna diversity being conserved and restored more successfully.

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Chapter 3

Effects of rewetting measures in Dutch raised bog remnants on assemblages of aquatic Rotifera and microcrustaceans

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◀ Decaying trunks of birch *Betula pubescens* that died after inundation of drained cut-over peat in the Mariapeel, The Netherlands.

Abstract

Species differ in their life cycle, habitat demands and dispersal capacity. Consequently different species or species groups may respond differently to restoration measures. To evaluate effects of restoration measures in raised bog remnants on aquatic microinvertebrates, species assemblages of Rotifera and microcrustaceans were sampled in 10 rewetted and 10 non-rewetted sites, situated in 7 Dutch raised bog remnants. A total of 129 species (Rotifera 108, Cladocera 15, Copepoda 6 species) were found. The species assemblages, total numbers of species and numbers of characteristic raised bog species did not differ between the 10 rewetted and 10 non-rewetted sites. The dominant pattern in the variation in microinvertebrate assemblages could be explained by the presence or absence of open water and variation in physico-chemical variables of surface water and organic matter. Furthermore, the species assemblages of water bodies situated in the same area were on average more similar to each other than to assemblages from other areas. These differences between areas may be due to differences in environmental conditions of water bodies, and possibly also to differences in the local species pool and the subsequent immigration sequence of species. We conclude that, in contrast to earlier findings on aquatic macroinvertebrates, populations of microinvertebrate species, including characteristic species, can either persist in the raised bog remnants during the process of rewetting or (re-)establish within a relatively short period of time (less than about five years).

3.1 Introduction

In degraded landscapes, restoration measures are taken to rehabilitate the ecosystems and their characteristic biodiversity. These measures focus mainly on creating suitable abiotic conditions for the recovery of key plant species or characteristic vegetation types. Recovery of characteristic fauna is often assumed to follow automatically in course of time. Although animals, and especially invertebrates, make up an important part of the total species diversity, relatively little attention has been paid to how restoration measures affect the fauna (Longcore 2003, Van Duinen et al. 2003, Van Kleef et al. 2006).

In raised bog landscapes *Sphagnum* species are key-stone species. Therefore, restoration measures in degraded raised bogs generally focus on creating suitable hydrological conditions for re-colonisation and growth of *Sphagnum* by blocking drainage ditches and building dams to retain rain water (e.g. Smolders et al. 2003, Rochefort et al. 2003, Vasander et al. 2003). These rewetting measures have been applied both in large-scaled cut-over bog remnants with little or no *Sphagnum* growth and in bog remnants degraded by drainage and small-scaled hand peat cuttings, where *Sphagnum* was still growing in parts of the area. Rewetting measures frequently resulted in a fast rise of the water table and shallow to deep flooding of large areas. The subsequent vegetation differed considerably between different rewetted sites. Floating rafts with *Sphagnum* vegetation developed if poorly humified peat was still present and swelled up or became buoyant. Submerged or floating *Sphagnum* vegetation developed if sufficient light penetrated into the (shallow) water layer and sufficient carbon dioxide was available. Inundated areas of humified peat remained almost devoid of *Sphagnum* (Smolders et al. 2003).

A comparative study on aquatic macroinvertebrates showed that rewetted sites in Dutch raised bog remnants are inhabited by only a part of the species spectra of both pristine raised bogs in Estonia and non-rewetted sites in Dutch bog remnants (Van Duinen et al. 2002 and 2003). These non-rewetted sites are water bodies that have not been subject to large-scale restoration measures, but are remnants of the former use of bogs, e.g. abandoned hand peat cuttings and

trenches used in buckwheat culture. The species assemblages differ considerably between rewetted and non-rewetted sites and the cumulative species richness is much higher in the non-rewetted sites. Moreover, several characteristic and rare species were found only at non-rewetted sites, irrespective of the development of a characteristic vegetation. These differences between rewetted and non-rewetted sites could not be explained by differences in the surface water quality and vegetation composition of the water bodies. The differences in species assemblage were attributed to fast and large-scaled changes in the water table caused by large-scaled rewetting measures and to lower habitat diversity in rewetted areas. Populations of rare and characteristic macroinvertebrate species, that were able to persist in degraded bog remnants, can respond negatively to rewetting of raised bog remnants (Van Duinen et al. 2003).

Contrary to these results on aquatic macroinvertebrates, Buttler et al. (1996) showed that the testate amoebae fauna of raised bogs can recover rapidly and fully, regardless of the initial condition of the cutover surface. Apparently, different invertebrate groups respond differently to restoration measures. This differential response may be explained by differences in dispersal capacity and habitat demands between invertebrate groups. Most aquatic macroinvertebrate species disperse actively. However, species may lack flight ability (e.g. Jackson 1952) or exhibit high site fidelity, which may especially be true for species living in stable and permanent environments (Southwood 1962, Wagner & Liebherr 1992), such as raised bogs. As a result, for macroinvertebrates re-colonisation may be very slow. Aquatic microinvertebrates, like Rotifera and small crustaceans (Copepoda and Cladocera), on the other hand, are known to be easily spread by wind and animal vectors (Cáceres & Soluk 2002, Cohen & Shurin 2003). Cohen & Shurin (2003) found that zooplankton (Rotifera, Copepoda and Cladocera) disperse rapidly over short distances (10m - 1km) and that dispersal only limits the diversity of very young communities (< 1 year). Furthermore, Rotifera, Copepoda and Cladocera species often reproduce parthenogenetically and have a short life cycle that can be completed in one water body (Nogrady et al. 1993, Wetzel 2001). Both these life-history characteristics potentially enable them to rapidly establish a population after colonisation. In contrast, most macroinvertebrates reproduce sexually and have a more complex life cycle than microinvertebrates that include larval, pupal, and adult stages. Each of these life-history stages may pose different demands on their environment. Therefore, a combination of various habitat elements may be needed to complete the life cycle (e.g. Galewski 1971, Fairchild et al. 2003). The various habitat elements (heterogeneity) required by the species to complete their life cycle have to be present at the proper time and proper spatial scale (Verberk et al. 2001 and 2005). In rewetted sites these requirements have apparently not been met for several macroinvertebrate species, contrary to the non-rewetted sites that were included in the macroinvertebrate study. This can be due to a more radical degradation (e.g., large-scaled peat extraction) in the rewetted sites, or to the abrupt process of rewetting, resulting in the disappearance of relic populations of rare and characteristic macroinvertebrate species (Van Duinen et al. 2003).

As Rotifera and microcrustaceans generally have a high dispersal capacity and a low dependence on habitat heterogeneity for the completion of their life cycle, compared to macroinvertebrates, we hypothesised that the occurrence of these microinvertebrate species, including characteristic raised bog species, do not differ between rewetted and non-rewetted sites in bog remnants. Contrary to the findings of the study on macroinvertebrates, we expected that physico-chemical conditions and vegetation structure of the water bodies are more discriminating factors between the microinvertebrate assemblages than conditions at a larger spatial scale or events in the past, including the degradation or rewetting of raised bog remnants. To test this hypothesis,

a comparative study was carried out, with a similar procedure as in the earlier study on macroinvertebrates (Van Duinen et al. 2003). We sampled Rotifera, Cladocera, and Copepoda in 10 water bodies created by rewetting measures and 10 water bodies in degraded raised bog remnants, which have not been subject to large-scale rewetting measures. These two groups of sampling sites will be referred to as 'rewetted sites' and 'remnant sites', respectively. To include variation in vegetation structure, each group consisted of 5 pools with a substantial area of open water and a littoral zone and 5 puddles with little or no open water. In this paper we will answer the following questions:

1. Do remnant sites differ from rewetted sites with respect to species assemblage and (cumulative) species richness?
2. Is there a different response for characteristic raised bog species?
3. What is the relative importance of rewetting, vegetation structure and physico-chemical variables for the species assemblages of Rotifera, Cladocera and Copepoda?

3.2 Materials and methods

Sampling sites

Microinvertebrates were sampled in 20 water bodies, situated in 7 different raised bog remnants in the Netherlands (Figure 1). Ten of these water bodies sampled were created by large-scale rewetting measures 5 to 30 years ago. The 10 remnant sites were pools and puddles created by peat cutting and trenches used in buckwheat culture. These remnant sites were abandoned more than 50 years ago and had not been subject to large-scale rewetting measures. The water bodies sampled differed in age, size and water and substrate quality. Characteristics of the sampling sites (year of rewetting, dimensions, and physico-chemical variables) are given in the appendix. Both the remnant and rewetted sites included five pools with a substantial area of open water in the centre and *Sphagnum cuspidatum* and other plants in the littoral zone, and five water bodies with little open water, viz. relatively small puddles and a trench used in buckwheat culture.

Microinvertebrate sampling and identification

To avoid large changes in species assemblages during the sampling period, all sites were sampled in the period of April 1 to May 6, 2002. At each sampling site two sets of subsamples were taken. Each set consisted of pooled subsamples collected at different spots in the water body. One set of subsamples was taken by means of two plankton nets with 45 mm and 115 mm mesh size, respectively, and was fixed immediately by adding formaldehyde to a final concentration of 2%. This concentration was later raised to 4% for storage. The other set of subsamples was taken using only a plankton net with 45 mm mesh size, was not fixed and was examined under the microscope to identify illoricate Rotifera. For the 10 pools, subsamples taken in the littoral zone were kept separately from those taken in the open water. Species were identified using the keys of Donner (1965), Koste (1978), Einsle (1993 and 1996), Nogrady (1995), Segers (1995), De Smet (1996 and 1997), and Flößner (2000). Hypochlorite solution was used for rotifer trophi analysis. Species lists are published by Zhuge & Van Duinen (2005).

Physico-chemical variables

At each sampling site, surface water was sampled without air-bubbles in iodated polyethylene bottles. Organic matter (top layer of sedimented detritus and accumulated peat) was collected using the plankton net with 115 mm mesh size. Both were sampled on the same day as the microinvertebrates. Electric conductivity (EC) and dimensions of the water bodies (length,

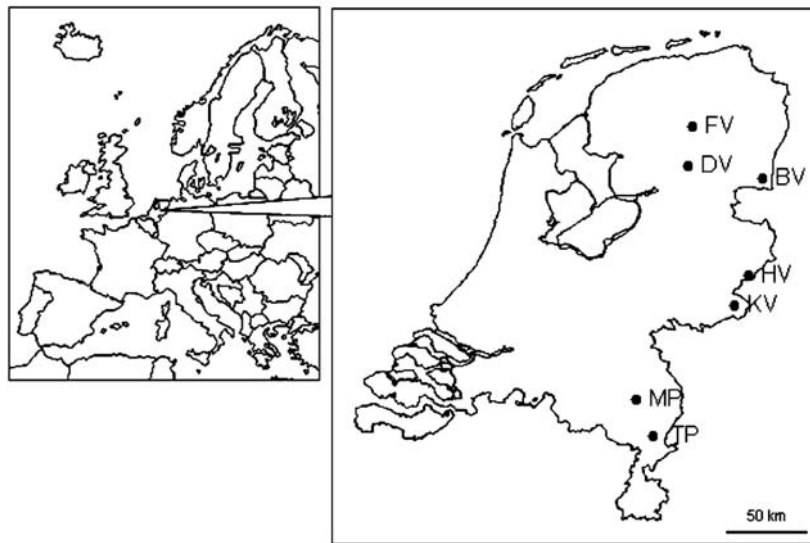


Figure 1. Locations of raised bog areas in the Netherlands at which samples were taken. FV=Fochteloërveen (0/2), DV=Dwingelerveld (2/0), BV=Bargerveen (1/3), HV=Haaksbergerveen (0/3), KV=Korenburgerveen (5/0), MP=Mariapeel (0/2), TP=Tuspeel (2/0). Figures in brackets are the numbers of remnant/rewetted sites sampled at each of the areas.

width and depth) were assessed at the sites. The pH, alkalinity and turbidity of surface water samples were measured within 24 hours after sample collection. After adding 1 mg citric acid per 5 ml of water, water samples were stored at -20°C in iodated polyethylene bottles until further analysis. Organic matter was dried (48 hours at 70°C) and ground up in liquid nitrogen. Nitrogen and carbon concentrations were measured in dried and ground organic matter with a CNS analyser (type NA 1500, Carlo Erba Instruments, Milan). Dried and ground organic matter was digested in sealed Teflon vessels in a Milestone microwave oven adding nitric acid and hydrogen peroxide. Digestates were analysed for total-P, Na, K, Ca, Cl, Mg, Mn, Fe, Si, Zn, Al, and S. The surface water samples were analysed for colour (extinction at 450 nm; Shimadzu spectrophotometer UV-120-01) and the concentrations of total inorganic carbon, NO₃, NH₄, PO₄, total-P, Na, K, Ca, Cl, Mg, Mn, Fe, Si, Zn, Al, and S. The following were determined colorimetrically: NO₃ according to Kamphake et al. (1967), NH₄ according to Grasshoff & Johannsen (1972), o-PO₄ according to Henriksen (1965), Cl according to O'Brien (1962). Na and K were determined with a Technicon flame photometer. Ca, Mg, Mn, Fe, Si, Zn, Al, P, and S were measured by inductively coupled plasma emission spectrophotometry. Total inorganic carbon in surface water was measured with an infrared carbon analyser. Selected water and organic matter quality data are presented in appendix 1.

Data analysis

Cumulative curves for species richness were compiled for both remnant and rewetted sites by calculating the averages and standard deviation of the total species richness of all possible combinations for one to ten sampling sites. Significance of differences in average species richness between rewetted and remnant sites was tested with a one-way ANOVA, in which the factor 'rewetting' was nested within the factor 'area' to account for possible differences in diversity between areas. Similarity in microinvertebrate assemblages of site pairs was calculated

using the Sørensen coefficient (Sørensen 1948) $S=2j/(a+b)$, with j being the number of species occurring in both sites, a and b the total species numbers of the two respective sites. Average similarity coefficients were compared within and between areas and between remnant and rewetted sites. Significance of differences in averages between categories of sites was tested using a Mann-Whitney-U test. Significance of differences in environmental variables between rewetted and remnant sites was tested with a one-way ANOVA.

Species were considered to be characteristic for raised bogs when they are listed as typical of bogs or acidophilous species (preferring acid water bodies) in literature (data in Zhuge & Van Duinen 2005). These data were taken from Myers (1931), Donner (1965), Koste (1978), Bērziņš & Pejler (1987), Pejler & Bērziņš (1993a), Nogrady & Pourriot (1995), Segers (1995), and De Smet (1996; 1997).

Multivariate analyses of individual species presence/absence data and environmental variables were performed in Canoco for Windows version 4.0 (Ter Braak & Šmilauer 1998), to study the relative importance of rewetting, open water and the physico-chemical variables mentioned in the previous paragraph. A first analysis was performed with the samples taken in puddles, in open water and in littoral zones of pools separately (30 samples). A second analysis was performed in which the species presence data from the open water and littoral zone of pools were pooled (10 samples taken in pools and 10 samples in puddles). From preliminary detrended correspondence analyses (DCA) it was concluded that most of the species response curves could be best described by a linear response model (following Ter Braak 1995). Therefore, principal components analyses (PCA) were performed for analysis of species presence data and environmental variables. Significance of the effect of environmental variables was tested using a Monte Carlo resampling procedure with 500 permutations in redundancy analyses (RDA). To test whether rewetting could significantly explain variation that remains after fitting the most important environmental variables, or the areas in which the sampling sites were situated, two partial RDAs were performed, with the binomial variable 'rewetting vs. remnant' as the only environmental variable and either the most important environmental variables, or 7 binomial dummy variables for the areas in which the sampling sites were situated, as covariables. This analysis was performed on the data set with littoral zone and open water samples pooled.

3.3 Results

Species richness and frequency

A total of 129 microinvertebrate species was found at the twenty sites sampled: 108 Rotifera, 15 Cladocera, and 6 Copepoda species. The remnant sites comprised 107 species and rewetted sites 95 species. The higher number of species in remnant sites mainly concerned non-characteristic species encountered in only one of the sampling sites (Figure 2). Most of the more frequently found species were present in both remnant and rewetted sites (non-lined parts in Figure 2). Fifteen species were encountered in 15 or more sites. These included the characteristic species *Acantholeberis curvirostris*, *Lecane galeata*, *Trichocerca parvula*, *Diacyclops nanus*, *Alonella exisa* and *Keratella serrulata*. 73 species were found at both rewetted sites and remnant sites, whereas 56 species were encountered at either the rewetted sites, or the remnant sites only. Of these 56 species, 34 were only found in the remnant sites, of which 7 species were classified as characteristic species. For the rewetted sites these numbers were 22 and 8, respectively. Eighty-five percent of these 56 species were encountered at only one or two sampling sites. Few species were encountered considerably more frequently at either remnant or rewetted sites. These were

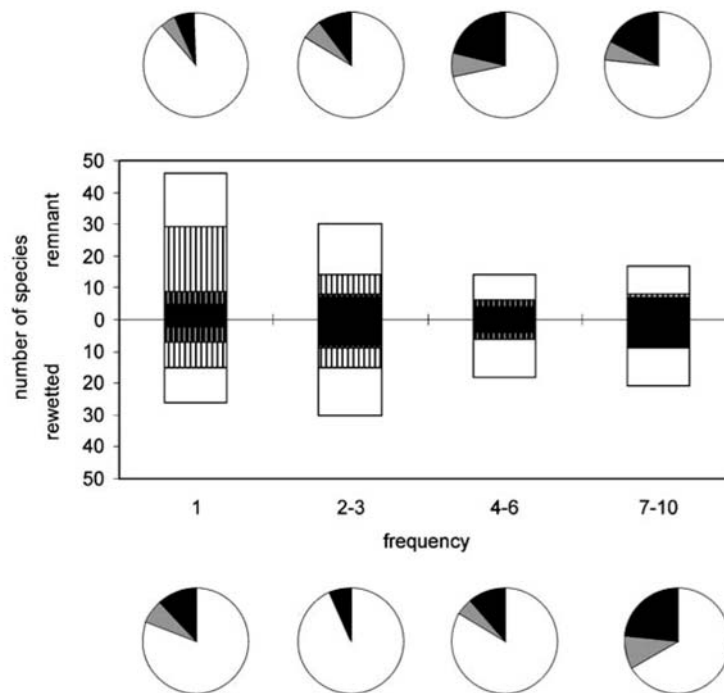


Figure 2. Numbers of species found at the 10 remnant sites (above horizontal axis) and the 10 rewetted sites (under horizontal axis) for different frequency classes. Characteristic species are shown with the black parts and non-characteristic species with the white parts. Species encountered in one of both groups of sites exclusively are shown with the lined parts of the black and white parts of the bars. The pie charts indicate the proportion of Rotifera (white), Copepoda (grey) and Cladocera (black) in the total number of species of the respective bars.

the characteristic Rotifera species *Pleurotrocha robusta* (5 remnant sites vs. no rewetted sites), *Monommata phoxa* (6 vs. 1), and *Euchlanis triquetra* (2 vs. 7), the rotifer *Encentrum martens* (7 vs. 0) and the copepod *Acanthocyclops robustus* (2 vs. 9).

The proportions of Rotifera, Copepoda and Cladocera in the total number of species were fairly similar between rewetted and remnant sites (Figure 2). The proportion of Cladocera was higher in the frequently encountered species than in the less frequently encountered species. In total, 38 species were classified as characteristic to acid water or bogs, of which 30 were recorded at the remnant sites and 31 at the rewetted sites. The average number of species and characteristic species did not differ significantly between remnant sites and rewetted sites (Table 1). The cumulative species richness of rewetted sites and remnant sites was similar, especially for the characteristic species (Figure 3).

Species assemblages and correlations

In the first PCA (Figure 4a) samples from rewetted and remnant sites were not separated along the first four PCA-axes. The distribution of samples along the first PCA-axis was positively or negatively correlated (with correlation coefficient <-0.5 or >0.5) to the Na and Cl concentration, electric conductivity, and pH of the surface water and to the C/N quotient of the organic matter.

Chapter 3

Table 1. Average ($\pm$ S.E.) species richness and numbers of characteristic species of the remnant and rewetted sites.

	Remnant sites	Rewetted sites	ANOVA
	n=10	n=10	
Species richness	42.5 $\pm$ 6.1	44.6 $\pm$ 6.3	n.s.
Characteristic species	15.2 $\pm$ 1.5	16.3 $\pm$ 2.1	n.s.

n: number of sampling sites; n.s.: not significant.

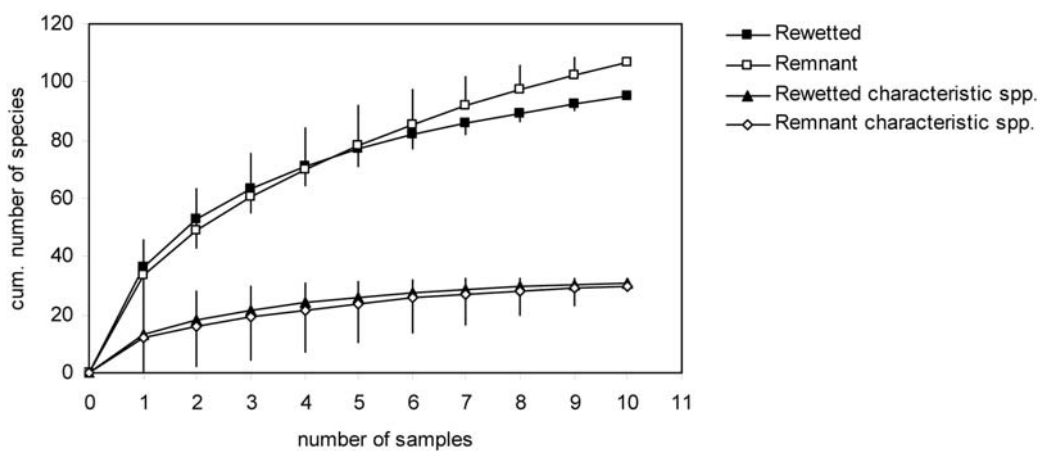


Figure 3. Cumulative species richness curves ($\pm$ standard deviation) for all species and characteristic species sampled in rewetted and remnant sites.

The open-water samples were separated from the samples taken in the littoral zone of pools and in puddles along the second PCA-axis. The first four PCA-axes explained 15.1%, 10.5%, 8.6%, and 7.7% of the variation in species data. After selecting all environmental variables that had a correlation coefficient <-0.5 or >0.5 with one or more of the first four PCA axes, the variables Na and o-PO₄ concentration and pH of the surface water, open water and 'rewetting vs. remnant' significantly (Monte Carlo procedure: $P<0.05$) explained the variation in species assemblages (Table 2). The binomial variable 'rewetting vs. remnant' explained 6.0% of the variation in species data, which is less than the variables pH, Na, o-PO₄ and open water, that explained 10.3%, 9.6%, 6.8% and 7.2%, respectively.

In the second PCA -with pooled species data of open water and littoral zone of pools- (Figure 4b) rewetted sites scored negatively and most remnant sites positively on the second PCA-axis, whereas pools and puddles were not separated along the first four PCA-axes. The first four PCA-axes explained 18.3%, 10.3%, 9.8%, and 8.9 % of the variation in species data. After selecting all environmental variables having a correlation coefficient <-0.5 or >0.5 with one or more of the first four PCA axes, the pH and the concentration of o-PO₄ in the surface water significantly (Monte Carlo procedure: $P<0.05$) explained the variation in species assemblages (Table 3). These variables explained 12.4% and 8.2% of the variation in species data, which is again more than the (non-significant) variable 'rewetting vs. remnant' that explained 7.8%.

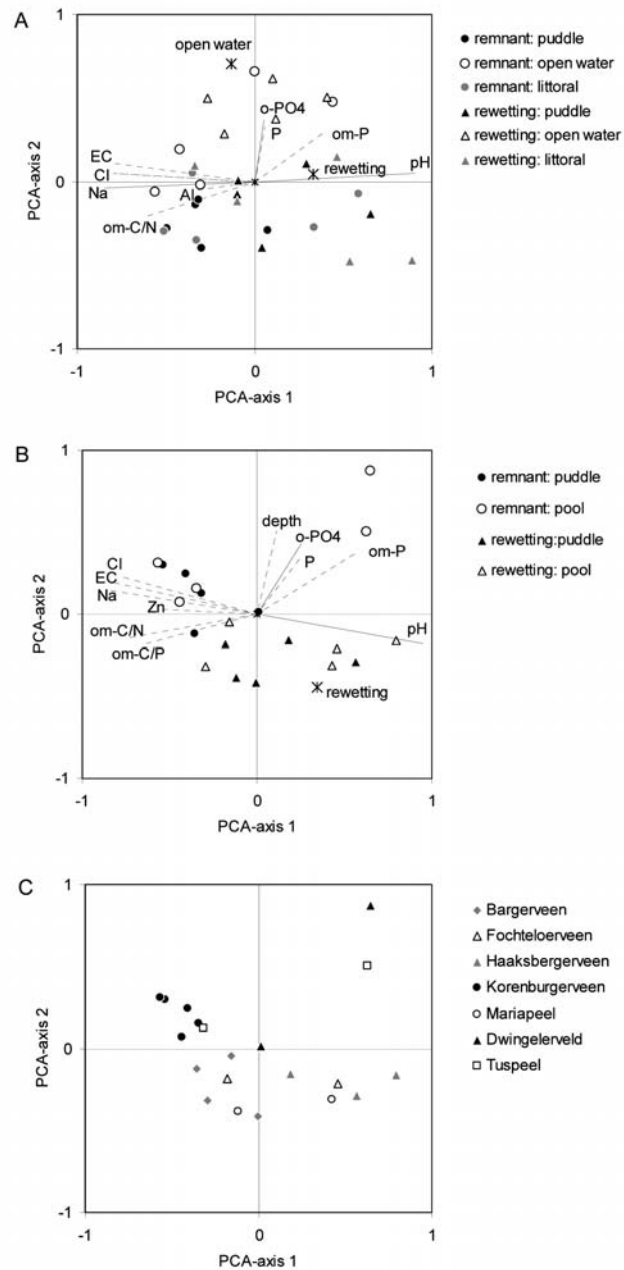


Figure 4. PCA-plots of samples based on species presence data. In figure A samples from open water and the littoral zone of pools are plotted separately, whereas they are pooled in B and C. Samples are categorised in remnant and rewetted sites (A and B) and C) in the area in which the sampled water bodies are situated. Significant explaining variables are shown with uninterrupted lines for continuous variables (length multiplied by 3) and an asterisk for centroids of binomial variables (score multiplied by 2). Interrupted lines are used for non-significant environmental variables having a correlation coefficient under -0.5 or above 0.5 with one or more of the first four PCA axes. 'om'=organic matter.

Chapter 3

Table 2. Eigenvalues (Lambda), P, and F-values of environmental variables having a correlation coefficient with one or more of the first four PCA axes of less than -0.5 or higher than 0.5 in the PCA of puddles, open water, and littoral zone samples (Figure 4a). P-values <0.05 are in italics.

	Marginal Effects	Conditional Effects		
	Lambda 1	Lambda A	P	F
pH surface water	0.10	0.10	<i>0.002</i>	3.21
Na surface water	0.10	0.04	<i>0.010</i>	1.72
Cl surface water	0.09	0.03	0.112	1.32
Electric conductivity surface water	0.08	0.03	0.475	1.01
Open water	0.07	0.07	<i>0.002</i>	2.34
o-PO4 surface water	0.07	0.07	<i>0.002</i>	2.15
P organic matter	0.07	0.04	0.174	1.25
P surface water	0.07	0.03	0.331	1.09
Rewetting	0.06	0.05	<i>0.010</i>	1.67
Al surface water	0.06	0.03	0.074	1.43
C/N quotient organic matter	0.06	0.03	0.293	1.13
Turbidity surface water	0.04	0.04	0.104	1.32
Ca organic matter	0.04	0.03	0.287	1.12

Table 3. Eigenvalues (Lambda), P, and F-values of environmental variables having a correlation coefficient with one or more of the first four PCA axes of less than -0.5 or higher than 0.5 in the PCA of puddles and pools, with open water and littoral zone samples pooled (Figure 4b). P-values <0.05 are in italics.

	Marginal Effects	Conditional Effects		
	Lambda 1	Lambda A	P	F
pH surface water	0.12	0.12	<i>0.002</i>	2.55
Na surface water	0.11	0.06	0.086	1.37
Cl surface water	0.10	0.04	0.607	0.85
P organic matter	0.10	0.04	0.601	0.93
EC surface water	0.10	0.03	0.679	0.78
Zn surface water	0.09	0.06	0.112	1.35
o-PO4 surface water	0.08	0.09	<i>0.044</i>	1.78
Rewetting	0.08	0.06	0.128	1.30
C/N quotient organic matter	0.08	0.05	0.421	1.04
P surface water	0.08	0.04	0.593	0.86
C/P quotient organic matter	0.07	0.03	0.737	0.81
Depth surface water	0.06	0.05	0.323	1.13

Table 4. Average Sørensen similarity index ($\pm$ S.D.) for pairs of sites situated in the same area and for pairs of sites in different areas; for pairs of rewetted sites and pairs of remnant sites; and for pairs of rewetted vs. remnant sites.

	n pairs	Sørensen-index	Mann-Whitney-U
in same area	23	0.64 $\pm$ 0.10	} <0.001
in different areas	167	0.51 $\pm$ 0.07	
remnant	45	0.56 $\pm$ 0.12	} n.s. } <0.05
rewetted	45	0.54 $\pm$ 0.08	
rewetted vs. remnant	100	0.51 $\pm$ 0.07	

The average concentrations of K, Ca, Fe and Si were significantly (one-way ANOVA: $P < 0.05$) higher at remnant sites. For the other environmental variables no significant differences were found. Rewetting could not significantly explain (Monte Carlo procedure: $P = 0.144$) the variation in species data that remained after fitting the environmental variables: open water, pH, o-PO_4 , Cl and Ca of surface water, and C/N quotient of organic matter.

The samples taken in the same raised bog remnant lie relatively close to each other in the PCA-plots (Figure 4c). This was most clear for the Korenburgerveen, Bargerveen, and Haaksbergerveen reserves. The average Sørensen similarity coefficients differed strongly (13%) between pairs of sites situated in the same area and pairs of sites in different areas ($P < 0.001$; Table 4). The difference was much smaller (4%), but still significant ($P < 0.05$), between the average similarity coefficient for pairs of rewetted vs. remnant sites and for pairs within the groups of rewetted sites and remnant sites. The average similarity coefficients did not differ significantly between pairs of remnant sites and pairs of rewetted sites. Rewetting could not significantly explain (Monte Carlo procedure: $P = 0.960$) the variation in species data that remained after fitting the 7 areas in which the sampling sites were situated.

3.4 Discussion

The average and cumulative number of characteristic Rotifera and microcrustacean species and species richness did not differ between remnant sites and rewetted sites. Differences in species assemblages between remnant sites and rewetted sites could be explained by physico-chemical variables and rewetting did not significantly explain remaining variation in the species data. All but eight of the species found in more than two sampling sites were found in both remnant and rewetted sites (For comparison, this number was 21 for macroinvertebrates, with 18 of these species only found in remnant sites and 3 only in rewetted sites). Only five species were encountered considerably more frequently at either remnant or rewetted sites. 82% of the characteristic microinvertebrate species were found in the rewetted sites and 79% in the remnant sites (These figures were 67% and 94%, respectively, for the macroinvertebrates).

Classification of aquatic microinvertebrates, especially Rotifera, as characteristic raised bog species is more disputable than for macroinvertebrates (Van Duinen et al. 2003), as less data on the distribution of microinvertebrate species are available. This can for example be illustrated by the many new Rotifera species records for New England presented by Błędzki & Ellison (2003) and by the 36 rotifer species encountered in our studies in Dutch bog remnants -from a total number of 129 species- that were not reported earlier from the Netherlands (Zhuge & Van Duinen 2005). Omitting species from or adding species to the list of characteristic species is not expected to considerably change the pattern described, as 89% of the species found in more than two sampling sites were found in both remnant and rewetted sites. Therefore, the conclusion that aquatic microinvertebrates species richness and number of characteristic species did not differ significantly between remnant and rewetted sites is not affected by the classification of characteristic species.

The relatively small difference in the microinvertebrate assemblage, compared to macroinvertebrates (cf. Van Duinen et al. 2003), between rewetted and remnant sites can be explained by both the less specific habitat demands and the high passive dispersal rate of microinvertebrates (Cáceres & Soluk 2002, Cohen & Shurin 2003). Regarding habitat demands, many Rotifera species -including many of those inhabiting raised bogs- are called ubiquitous

or eurytopic, as they occur in a broad range of pH values (Bērziņš & Pejler 1987) and are not very specific in their substrate choice (Pejler & Bērziņš 1993b, 1993c and 1994). In total 46 of the species found in the Dutch raised bog remnants were classified as characteristic species, but within bogs most species can be found in various ecotopes (Pejler & Bērziņš 1993a, Jersabek 1995). Eight of the characteristic species, as well as 14 non-characteristic species, were found in more than 50% of the water bodies sampled in the present study. However, some species prefer more wet ecotopes, whereas others can tolerate the dryer parts (cf. Bateman & Davis 1980, Pejler & Bērziņš 1993a).

The difference in species assemblages between open water versus puddles and the littoral zone of pools found by us (Figure 4a), is in accordance with results of the study by De Goeij (1987) who found the presence or absence of open water to be a main factor for differences in the species assemblages in Irish bog pools. About 80% of the Rotifera species found in our sampling sites were sessile, benthic or periphytic and thus in need of macrophytes or other substrates, whereas about 10% of the Rotifera species encountered were planktonic (data not shown).

Next to the presence or absence of open water, the Na, Cl, Zn, o-PO₄ and total-P concentration, pH, and electric conductivity of surface water and the P content and C/N and C/P quotient of organic matter were correlated to the variation in species assemblages (Figure 4). Variation in Rotifera and microcrustacean assemblages is commonly found to be correlated to the trophic state of water bodies (Pejler & Bērziņš 1989, Duggan et al. 2002). Epiphytic, sedimented or suspended algae, decomposing organic matter and microbes are important food sources for the groups of microinvertebrates studied (Pejler 1983). Dissolved P and N are well known as important factors in the production of algae (Wetzel 2001), whereas the N and P contents of organic matter and pH are important factors in its decomposition rate and the microbial activity (Kok & Van de Laar 1991, Belyea 1996, Smolders et al. 2002), besides the phenolic content of the organic matter (Kok et al. 1992, Kok & Van der Velde 1994). Nutrient availability and organic matter quality could also explain the occurrence of algivorous and detritivorous aquatic oligochaete species in raised bogs (G.A. van Duinen, unpublished data). Differences in the availability of minerals may also play a role in food quality. The higher average concentration of K, Ca, Fe and Si in remnant sites may indicate a larger influence of minerotrophic ground water in several of the remnant sites. It was beyond the scope of this study to completely unravel causal relations between species assemblages and environmental conditions. This requires more direct measurements on food quality and availability, information on nutritional requirements and food preferences of the species and probably data on food web structure. The present study showed that environmental variables related to vegetation structure and food quality and availability could explain the dominant pattern in the variation in microinvertebrate assemblages.

Species assemblages of water bodies situated in the same area were found to be more similar to each other than to assemblages from other areas (Table 4). This was most clear for the Korenburgerveen, Bargerveen, and Haaksbergerveen reserves where three to five water bodies were sampled (Figure 4c). Apart from the Bargerveen reserve, the sites sampled in one area were all either rewetted or not rewetted. It is unlikely that rewetting is the key factor in these differences between areas, as the Sørensen similarity index differed much stronger between areas than between rewetted vs. remnant. The differences in species assemblages between areas were correlated to several environmental variables (Figure 4b) and might be explained by local factors affecting water quality and food availability, such as minerotrophic influence, quality of the peat substrate and atmospheric nitrogen deposition. Another explanation for the strong

differences in the species assemblages between areas may be the differences in the local species pool and interspecific interactions (Shurin et al. 2000). For instance, twenty species were found in all or most of the four and five sites sampled in Bargerveen and Korenburgerveen, but the rotifers *Monommata phoxa*, *Pleurotrocha robusta* and *Lecane hamata* and the copepod *Acanthocyclops robustus* were found to be frequent in one of both areas and absent in the other area (data in Zhuge & Van Duinen 2005), whereas no clear differences were found in the environmental variables between these areas (Appendix). Species already abundant in a bog remnant will be among the first to colonise adjacent, new water bodies created by peat-cutting in the past and more recently by rewetting measures. These ‘early’ species have a high chance to successfully establish a population. Species arriving later may generally have a lower chance to establish a population, as interactions with resident species may prevent them from increasing (Jenkins & Buikema 1998, Shurin 2000, Rundle et al. 2002, Cohen & Shurin 2003).

In conclusion, the species richness, occurrence of characteristic species, and species assemblages of Rotifera, Copepoda and Cladocera did not differ between rewetted and non-rewetted sites in raised bog remnants. The variation in species assemblages could be explained by variation in physico-chemical variables and the presence of open water or vegetation in the water bodies. Contrasting to macroinvertebrate populations, that may experience a severe drawback from large-scaled rewetting measures (Van Duinen et al. 2003), populations of microinvertebrate species, including characteristic species, were not negatively affected by large-scale rewetting. Populations of microinvertebrate species were able to persist during the abrupt process of rewetting or to (re-)establish within a short period of time. At a site rewetted five years before sampling, 17 characteristic species were found, which is higher than the average for remnant and rewetted sites (Table 1). Although we did not study causal relations between species occurrence and environmental variables in the raised bog remnants, the microinvertebrate assemblage is likely structured by the environmental conditions at the scale of the water body and possibly also by the local species pool and interspecific interactions. Of course, also macroinvertebrate assemblages are structured by environmental conditions. However, many macroinvertebrates complete their life cycles on larger scales in space and time. They may require different habitat elements in different life-history stages (Verberk et al. 2005). Differences in the life cycles of species, as well as differences in the dispersal capacity could explain their differential response to restoration measures in raised bog remnants and other landscape types as well.

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Appendix

Selected environmental variables for the sampling sites

	Bargerveen				Fochteloër-veen			Haaksbergerveen			Korenburgerveen					Mariäpeel		Dwingeler-veeld		Tuspeel	
	1	2	3	4	1	2	3	1	2	3	1	2	3	4	5	1	2	1	2	1	2
Rewetting	-	1970	1975	1995	1986	1990	1970	1970	1970	1970	-	-	-	-	-	1990	1996	-	-	-	-
Pool/Puddle	Pu	Pu	Po	Po	Pu	Pu	Pu	Pu	Pu	Pu	Pu	Pu	Pu	Po	Po	Pu	Po	Pu	Pu	Po	Pu
Depth (m)	0.15	0.2	0.2	0.5	0.25	0.2	0.2	0.2	0.9	0.9	0.33	0.35	1.5	1.5	1.5	0.5	1.3	1	1	1.3	0.05
Width (m)	0.3	1	12	100	100	1	1.5	1	30	30	3.5	1	10	2	8	0.4	50	5	3	10	0.3
Length (m)	100	4	30	100	300	20	50	10	80	80	5	5	10	5	8	1.5	100	10	15	15	0.8
Surface water																					
Turbidity (ppm)	10	24	9	17	23	13	12	10	11	11	25	15	13	17	13	23	20	8	10	10	12
pH	3.9	4.1	3.8	3.9	4.1	4.2	4.1	4.1	4.0	4.0	3.8	3.8	3.7	3.7	3.9	4.4	4.3	4.7	4.1	4.3	4.4
EC ($\mu\text{S.cm}^{-1}$)	75	40	75	93	58	65	50	50	53	53	80	75	124	94	62	75	65	65	57	57	85
Cl ($\mu\text{mol.l}^{-1}$)	288	185	279	337	282	255	162	158	181	181	269	301	361	333	328	165	223	273	225	145	187
Na ($\mu\text{mol.l}^{-1}$)	278	307	312	327	302	268	161	146	166	166	285	324	341	327	370	224	263	263	241	207	222
o-PO ₄ ($\mu\text{mol.l}^{-1}$)	1.5	1.7	1.2	3.9	0.9	0.8	1.1	0.5	0.6	0.6	0.9	1.2	1.5	0.9	1.1	1.0	1.1	6.5	0.5	1.4	0.4
NO ₃ ($\mu\text{mol.l}^{-1}$)	3.2	2.5	3.0	3.0	1.8	2.4	3.2	3.4	5.2	5.2	19.5	6.0	12.6	4.7	5.4	2.4	3.0	1.1	2.6	3.6	3.5
NH ₄ ($\mu\text{mol.l}^{-1}$)	92.4	4.9	8.7	7.6	6.9	8.2	1.2	2.1	27.2	27.2	44.4	37.4	5.1	9.6	6.9	5.4	12.6	4.4	6.1	6.7	8.2
Ca ($\mu\text{mol.l}^{-1}$)	26	21	29	26	30	35	26	20	28	28	58	47	59	46	43	17	43	33	53	30	48
Zn ($\mu\text{mol.l}^{-1}$)	218	427	214	206	10	32	18	20	24	24	265	203	202	243	267	164	215	8	16	329	190
total-P ($\mu\text{mol.l}^{-1}$)	1.6	4.7	1.4	5.6	1.5	1.2	1.8	0.9	0.8	0.8	1.3	1.6	1.8	1.3	1.7	1.6	2.8	8.9	0.9	2.3	1.3
Al ($\mu\text{mol.l}^{-1}$)	17.5	4.9	9.7	6.9	4.1	4.9	9.3	8.5	9.3	9.3	10.9	10.3	11.1	8.2	8.5	5.7	9.1	2.1	4.4	8.1	8.5
Organic matter																					
Ca ($\mu\text{mol.g}^{-1}$ DW)	46	34	52	79	78	60	145	51	61	61	29	5	58	62	58	77	37	16	64	44	47
total-P ($\mu\text{mol.g}^{-1}$ DW)	7	10	17	7	19	5	32	16	26	26	9	17	20	21	20	35	29	63	12	14	13
C/N quotient (g.g^{-1})	48	38	24	40	23	28	18	17	17	17	37	26	26	21	26	19	15	14	14	21	47
C/P quotient (g.g^{-1})	4514	3352	1774	4514	1591	1081	962	1063	1214	1214	3605	1929	1986	1526	1986	903	979	473	558	1621	2269

-: no rewetting measures.



Chapter 4

Pristine, degraded and rewetted bogs: Restoration constraints for aquatic macroinvertebrates

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Submitted

◀ Sampling of aquatic invertebrates at a pristine bog pool in Nigula nature reserve, Estonia.

Abstract

Raised bog landscapes are degraded by reclamation, causing loss of natural gradients between ombrotrophic bog massifs and the surrounding minerotrophic landscape. In addition, bogs are vulnerable to nutrient enrichment. To restore degraded bogs rainwater is retained, aiming at recovery of a *Sphagnum*-dominated vegetation. We hypothesized that increased nutrient availability enables macroinvertebrate species naturally absent from extremely nutrient limited bog massifs to become abundant in nutrient enriched bog remnants. Our second hypothesis was that macroinvertebrate assemblages characteristic of gradients from ombrotrophic bogs to minerotrophic surroundings hardly profit from the restoration practice focusing on ombrotrophic conditions solely. The aquatic macroinvertebrate abundance was higher in bog remnants in The Netherlands, where nitrogen and phosphorus concentrations are increased, than in ombrotrophic water bodies and in transitional mires in pristine reference systems in Estonia. This increase was indeed primarily due to species that were absent from Estonian bogs and species preferring transitional mires. In water bodies created by rewetting measures the abundance of species preferring nutrient poor ombrotrophic pools was higher than in water bodies remaining after historical use of bogs, such as peat cutting pits. The cumulative richness of species preferring nutrient poor ombrotrophic pools was however not increased and cumulative species richness and abundance of species preferring more minerotrophic parts of bog gradients were decreased. In contrast, remnant sites included the whole gradient from acid water bodies in bog massifs to the lagg of complete raised bog landscapes. Further reduction of nitrogen and phosphate availability in bogs and restoration of transitional habitats in and adjacent to remaining bog remnants, as well as in fen reserves currently including relict populations of species typical for bog gradients is recommended.

4.1 Introduction

Raised bog landscapes are characterised by both an ombrotrophic bog massif that is acid and extremely nutrient limited as well as gradients from the bog massif to the more minerotrophic surroundings, where these harsh conditions are gradually attenuated (Wheeler & Proctor 2000). Both elements are threatened due to human activities (Hogg et al. 1995, Joosten & Clarke 2002). In the European Union, active raised bogs, degraded raised bogs still capable of natural regeneration, and transition mires and quaking bogs are listed as habitat types H7110, H7120, and H7140 in Annex 1 of the Habitats Directive, with H7110 listed as priority habitat type (European Council 1992). At present the conservation status of these habitat types is evaluated as “bad” or “inadequate” in many European countries (European Environment Agency 2012). Past and current restoration efforts in degraded bog remnants in Europe and North America have been mainly focused on rewetting by means of retention of rainwater, aiming at regeneration of ombrotrophic *Sphagnum*-dominated vegetation (Meade 1992, Wheeler & Shaw 1995, Rochefort et al. 2003), as *Sphagnum* is a keystone genus in raised bogs (Rochefort 2000). Beyond *Sphagnum*, much less is known how macroinvertebrate species characteristic of ombrotrophic and more minerotrophic elements of raised bog landscapes have responded to degradation. Such knowledge is essential for evaluating restoration measures (Van Duinen et al. 2003, Verberk et al. 2010a). Based on knowledge of the process of degradation we forward two complementary hypotheses.

Ombrotrophic raised bog massifs have suffered from increased availability of nutrients. Both nitrogen (N) and phosphorus compounds (P) have increased dramatically (Van Duinen et al. 2006a; see also Table 1 in this study reporting a 4-6 fold increase), resulting from atmospheric deposition (Tomassen et al. 2004) and increased peat mineralisation (Lamers et al. 1999). Effects

of N-deposition on *Sphagnum* mosses and other bog plants have been intensively studied, showing significant changes in plant species composition and N-content of mosses and vascular plants (e.g. Aerts et al. 1992, Lamers et al. 2000, Berendse et al. 2001, Bragazza et al. 2005, Limpens et al. 2011). In contrast, data is largely lacking on the responses of macroinvertebrates to eutrophication. Increased nutrient availability may facilitate the invasion of certain macroinvertebrate species that are naturally excluded from the extremely nutrient limited raised bog massif, or only present in low abundance, to become abundant in nutrient enriched raised bog remnants (Hypothesis 1).

Gradients from the minerotrophic surroundings to the bog massif, including lagg zones and transitional mires have suffered extensive losses. The reason for this is that degradation processes (e.g. drainage, peat extraction, cultivation and afforestation) started at the margins of bog landscapes (Van den Munckhof 1993, Wheeler & Shaw 1995). Remnants of former raised bog landscapes therefore comprise predominantly parts of the former ombrotrophic bog massif. Where gradients from the bog massif to the surrounding landscape are still present, their nature quality is impacted by a loss of groundwater due to drainage of the surrounding landscape (Schouwenaars 1993, Baaijens et al. 2011). Thus, the minerotrophic parts of raised bog landscapes have become rare and severely degraded. Consequently, endangered species preferring these minerotrophic parts of intact gradients in bog landscapes are scarce or not recorded from bog remnants in The Netherlands (Van Kleef et al. 2012). Former lagg zones and transitional mires are generally outside the boundaries of the present-day bog reserves. Therefore, these elements of a raised bog landscape are rarely the focus of restoration efforts. Large-scale rewetting of degraded bog remnants by rainwater retention may even decrease the remaining influence of groundwater resulting in a loss of heterogeneity and a homogenization of aquatic macroinvertebrate assemblages (Verberk et al. 2010a). Consequently, it is hypothesised that species assemblages characteristic of lagg and transitional mires, including endangered species, hardly profit from those restoration efforts that focus exclusively on restoring ombrotrophic conditions (Hypothesis 2).

The above hypotheses may seem mutually exclusive, but this is not necessarily the case. Out of all species excluded from pristine bog massifs, some will be excluded because of low nutrients and these will be expected to profit from eutrophication in bog remnants, irrespective of restoration efforts being taken (Hypothesis 1). In contrast, the requirement for minerotrophic elements likely concerns other species and these will have declined in bog remnants, without profiting from restoration efforts (Hypothesis 2). To test both hypotheses, we compared the aquatic macroinvertebrate species composition of water bodies in raised bog remnants in The Netherlands and reference raised bog systems in Estonia. The sampled water bodies in Dutch bog remnants comprised two types of water body. The first type of water bodies, referred to as remnant sites, were remnants of former peat cuttings and trenches, abandoned in the first half of the twentieth century in The Netherlands. The other type of water body, referred to as restoration sites, were water bodies created by rewetting efforts, aimed at the restoration of raised bog habitats. The Estonian bog systems were only marginally influenced by human activities and out of all pristine raised bog landscapes on the European mainland they were among the closest to The Netherlands, with an almost identical species pool (Illies 1978, Fauna Europaea 2011). By taking the Estonian raised bog systems as a reference condition, we were able to ask how species characteristic of ombrotrophic bog massifs, transitional mires, or lagg zones in a pristine bog landscape have responded to degradation and restoration, contrasting the abundance and richness of these species differing in habitat use.

Chapter 4

4.2 Materials and methods

Study areas

Sampling included most types of water body in both pristine raised bogs (Smits et al. 2002) and Dutch raised bog remnants (Van Duinen et al. 2003). In total 31 water bodies were sampled in raised bog landscapes in Estonia; 27 of these water bodies were located in Nigula Nature Reserve, two in Valgeraba, Soomaa National Park, and two in Punaraba, Endla Nature Reserve (Figure 1). These 31 sites were classified in three types of water body. One type included 13 ombrotrophic bog pools with stagnant acid and nutrient poor water. The second type included 15 sites that were also acid and dominated by *Sphagnum* mosses, but had an increased availability of minerals and nutrients. In seven sites this increased availability resulted from flow of acid water from the mire expanse to the bog margin, or from springs of upwelling acid water from

Table 1. Chemical and physical data of the sampling sites in the distinguished types of water body in raised bog landscapes in Estonia and in the remnant and restoration sites in bog remnants in The Netherlands. Means ($\pm$ standard error) are presented for chemical variables and minimum and maximum values for physical variables. The numbers of sampling sites with open water and temporal water bodies are presented at the bottom of the table. n= number of sampling sites.

	ombrotrophic (stagnant) n=13	Estonia water flow + transitional mire n=15	lagg n=3	The Netherlands remnant n=33	restoration n=32	P
Surface water						
pH	3.9 $\pm$ 0.05 ^a	4.3 $\pm$ 0.14 ^b	5.5 $\pm$ 0.50 ^b	4.1 $\pm$ 0.08 ^{ab}	4.1 $\pm$ 0.05 ^{ab}	**
NO ₃ ⁻ +NH ₄ ⁺ (μ mol l ⁻¹)	6.2 $\pm$ 1.8 ^a	6.7 $\pm$ 1.3 ^a	3.2 $\pm$ 0.5 ^a	38.6 $\pm$ 7.2 ^b	35.4 $\pm$ 6.8 ^b	***
o-PO ₄ ³⁻ (μ mol l ⁻¹)	1.1 $\pm$ 0.3 ^{ab}	0.8 $\pm$ 0.2 ^a	0.8 $\pm$ 0.2 ^{ab}	6.4 $\pm$ 5.0 ^{ab}	3.4 $\pm$ 0.8 ^b	**
S (μ mol l ⁻¹)	18.6 $\pm$ 1.9 ^a	17.8 $\pm$ 2.4 ^a	17.8 $\pm$ 4.1 ^{ab}	52.9 $\pm$ 4.6 ^b	51.1 $\pm$ 2.5 ^b	***
Cl ⁻ (μ mol l ⁻¹)	71.5 $\pm$ 7.9 ^a	72.3 $\pm$ 4.6 ^a	76.0 $\pm$ 6.5 ^{ab}	271.2 $\pm$ 14.6 ^{bc}	332.9 $\pm$ 18.3 ^c	***
Ca ²⁺ (μ mol l ⁻¹)	48.1 $\pm$ 7.3 ^{ab}	61.2 $\pm$ 11.9 ^{ab}	156.7 $\pm$ 14.4 ^b	42.4 $\pm$ 4.2 ^a	42.8 $\pm$ 4.8 ^a	*
Interstitial water						
pH	4.5 $\pm$ 0.1	4.8 $\pm$ 0.1	5.9 $\pm$ 0.4	4.7 $\pm$ 0.1	4.7 $\pm$ 0.1	ns
NO ₃ ⁻ +NH ₄ ⁺ (μ mol l ⁻¹)	17.4 $\pm$ 6.1 ^a	9.8 $\pm$ 2.0 ^a	12.5 $\pm$ 7.7 ^{ab}	113.3 $\pm$ 23.1 ^{bc}	142.5 $\pm$ 24.1 ^c	***
o-PO ₄ ³⁻ (μ mol l ⁻¹)	0.7 $\pm$ 0.2 ^a	1.0 $\pm$ 0.2 ^a	3.0 $\pm$ 1.9 ^{ab}	13.4 $\pm$ 6.3 ^b	25.7 $\pm$ 8.8 ^b	***
S (μ mol l ⁻¹)	21.1 $\pm$ 4.0 ^a	21.2 $\pm$ 2.9 ^a	24.9 $\pm$ 1.6 ^{ab}	92.2 $\pm$ 8.8 ^b	80.3 $\pm$ 8.2 ^b	***
Cl ⁻ (μ mol l ⁻¹)	65.0 $\pm$ 3.9 ^a	84.8 $\pm$ 6.2 ^a	124.4 $\pm$ 22.8 ^{ab}	341.3 $\pm$ 38.2 ^b	420.5 $\pm$ 33.8 ^b	***
Ca ²⁺ (μ mol l ⁻¹)	59.7 $\pm$ 11.2 ^a	83.0 $\pm$ 17.8 ^{ab}	994.4 $\pm$ 747.1 ^b	108.3 $\pm$ 12.6 ^{ab}	104.7 $\pm$ 14.6 ^{ab}	*
Organic matter						
Ca ²⁺ (μ mol g ⁻¹ DW)	48.4 $\pm$ 2.7 ^{ab}	95.7 $\pm$ 15.8 ^b	197.9 $\pm$ 82.2 ^b	47.5 $\pm$ 4.8 ^a	70.1 $\pm$ 10.1 ^{ab}	***
P (μ mol g ⁻¹ DW)	16.1 $\pm$ 1.5	21.2 $\pm$ 1.5	30.8 $\pm$ 6.5	22.9 $\pm$ 2.1	19.9 $\pm$ 2.0	ns
S (μ mol g ⁻¹ DW)	116.4 $\pm$ 8.4 ^{ab}	104.9 $\pm$ 8.4 ^a	89.2 $\pm$ 24.0 ^{ab}	193.4 $\pm$ 15.2 ^b	174.3 $\pm$ 18.6 ^{ab}	**
C:N quotient g g ⁻¹	31.4 $\pm$ 2.9	33.4 $\pm$ 3.0	17.9 $\pm$ 1.6	24.9 $\pm$ 1.7	27.3 $\pm$ 1.9	*
C:P quotient g g ⁻¹	1155 $\pm$ 99 ^b	863 $\pm$ 75 ^{ab}	342 $\pm$ 114 ^a	920 $\pm$ 83 ^{ab}	976 $\pm$ 104 ^{ab}	*
Depth (cm)	13.0 – 250.0	4.0 – 250.0	15.0 – 20.0	0.1 – 70.0	0.1 – 75.0	
Area (m ²)	8.0 – 1500	0.3 – 175,000	4.0 – 30	0.0 – 5000	0.2 – 420,000	
Water flow (cm s ⁻¹)	0 – 0	0 – 18.0	0 – 4.0	0 – 0	0 – 0	
Open water (# sites)	12	9	0	24	27	
Temporal (# sites)	0	6	3	17	4	

Significance of the differences: ns = p>0.05, * = p<0.05, ** = p<0.01, and *** = p<0.001. Different letters indicate significant differences between habitat categories.

a deeper peat layer. In the other eight sites of this type of water body this increased availability of minerals and nutrients was a result of buffered groundwater, which was apparent from the occurrence of plant species like *Phragmites australis*, *Menyanthes trifoliata*, *Carex lasiocarpa*, or *Carex rostrata*. These vascular plant species are characteristic for transitional mires, where they occur in combination with *Sphagnum* mosses. Finally, the third type of water body included three sampling sites situated in lags. Physical and chemical characteristics of these types of water body are presented in Table 1.

In The Netherlands, samples were taken from 65 water bodies in eight raised bog remnants (Figure 1). These water bodies differed in size, water and organic matter quality, and composition and structure of the vegetation. In total 32 of these water bodies were restoration sites, created by inundation of either large-scale peat extraction fields or small-scale mosaics of hand peat cutting pits. They differed in peat quality and vegetation composition before the water level was raised by retention of rain water one to thirty years before sampling. The 33 remnant sites sampled were situated in small-scale mosaics of water-filled hand peat cuttings and dry peat

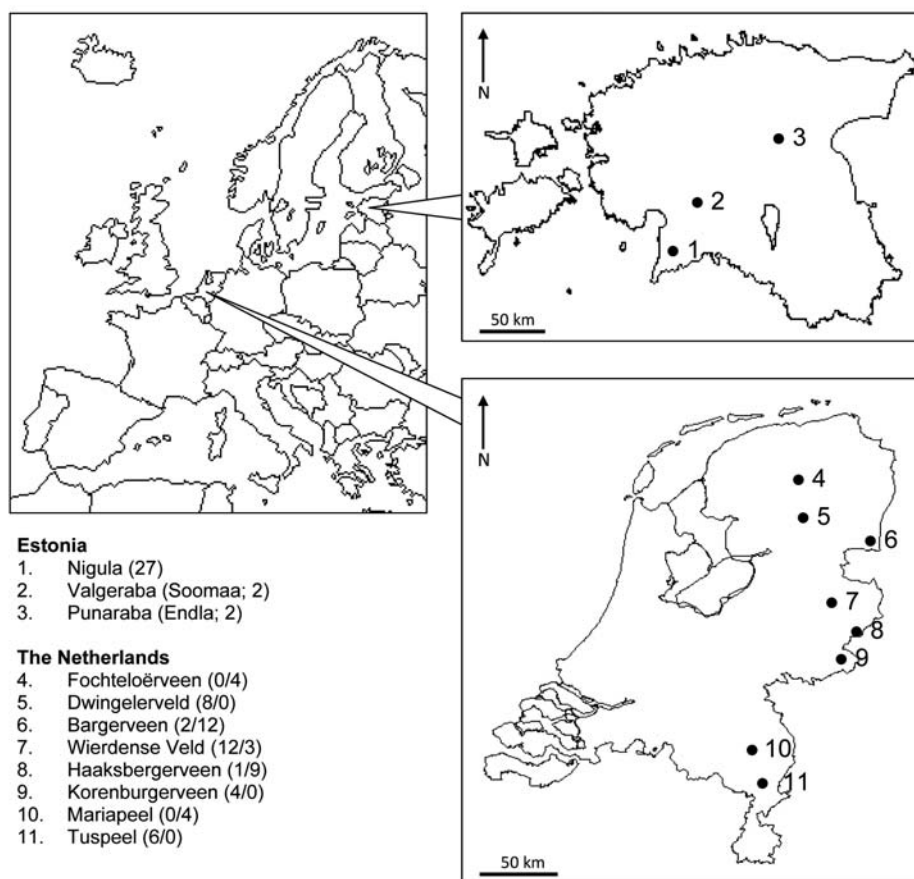


Figure 1. Location of study areas in Estonia and The Netherlands. The numbers of sampling sites in each area are presented between brackets. For the Dutch areas the numbers of remnant sites/rewetted sites are given.

strips, or trenches formerly used in buckwheat (*Fagopyrum esculentum*) culture. These sites were abandoned before 1950 and had not been subject to inundation. These remnant sites were in different stages of secondary succession.

Macroinvertebrate sampling

Macroinvertebrates were sampled using a 20x30 cm pond net with 0.5 mm mesh size. Most samples consisted of a 1 m sweep from more or less open water into more dense vegetation near the shore. If the water body only included open water, one or more longer sweeps were taken. In very dense *Sphagnum* vegetation, shorter sweeps were made to avoid the pond net becoming clogged with *Sphagnum*. As fauna abundance is generally high in this dense vegetation, most of these small samples contained over 100 individuals, or even more than 1000. Species abundance data per metre pond net sweep from spring and autumn samples were summed and divided by two, except for the twelve temporary remnant water bodies that could only be sampled in spring.

The Estonian sites were sampled both in spring 2001 and autumn 2005. The Dutch sites were also sampled in spring (1999, 2003 or 2006) and autumn (1998, 1999, 2003 or 2006), except twelve temporary remnant water bodies which were dry in autumn and therefore were only sampled in spring. Samples were transported to the laboratory and stored at 4°C until analysis. The collected material was washed over three sieves (2, 1, and 0.5 mm mesh sizes, respectively) and sorted in white trays. Oligochaeta, nymphs of Odonata, adults and nymphs of Heteroptera, larvae of Chironomidae, Chaoboridae and Trichoptera, and adults of Coleoptera were identified to species level if possible. Other life stages and groups of macroinvertebrates were not included in the analyses.

Environmental variables

In Estonia dead organic matter (top layer of accumulated peat and deposited detritus), interstitial water, and surface water were collected in May and June 2001 and in September 2005. At the Dutch sampling sites surface water and interstitial water were sampled one to four times between October 1998 and October 2006. Dead organic matter was collected once in August 1999, April/May 2003, or May 2006. Nitrogen and carbon concentrations were measured in dried organic matter samples. Digestates of ground-dried organic matter were analyzed for total-P, Na, K, Ca, Cl, Mg, Mn, Fe, Si, Zn, Al, and S. The surface and interstitial water samples were analysed for pH, alkalinity, colour (extinction at 450 nm) and the concentrations of total inorganic carbon, nitrogen (NO_3 and NH_4), PO_4 , total-P, Na, K, Ca, Cl, Mg, Mn, Fe, Si, Zn, Al, and S. For methods of sampling and analyses see Van Duinen et al. (2003, 2006a).

Data analysis

To test for the major differences in environmental conditions between the three types of sampling sites in Estonia (classified on the basis of the vegetation, see 2.1) and the remnant and restoration sites in The Netherlands, we performed a Kruskal–Wallis one-way analysis of variance with pairwise comparisons in SPSS version 19. Statistical significance of differences in macroinvertebrate species richness and log transformed total species abundance between the five categories of sampling sites was tested with a one-way analysis of variance with a Tukey HSD post-hoc test.

To describe the variation in macroinvertebrate species composition within and between the five different types of water body we performed a Correspondence Analysis (CA) of log transformed species abundance data using Canoco for Windows version 4.0 (Ter Braak & Smilauer 1998).

A preliminary detrended correspondence analyses (DCA) showed that species response curves could be best described by an unimodal response model and therefore a CA was performed (following Ter Braak 1995). Significance of the effect of environmental variables was tested using a Monte Carlo resampling procedure with 500 permutations in a Canonical Correspondence Analysis (CCA). Fifteen of the 214 species recorded in this study were not included in this analysis as either Estonia or The Netherlands were outside their distribution area (according to our study and Fauna Europaea (2011)).

To analyse how species characteristic of the different elements in a pristine bog landscape have responded to degradation and restoration, we classified the aquatic macroinvertebrate species based on their abundance in three parts of pristine raised bog landscapes in Estonia into three habitat use categories: 1) ombrotrophic water bodies, 2) water bodies on the gradient to the margin of the bog, including bog brooklet, marginal slope, transitional mire, and 3) the lagg zone. Criteria for the classification of species in habitat use categories are presented in Table 2. Subsequently, the abundance, average species richness and total (cumulative) richness of the species in each of these three habitat use categories was compared across the 5 types of water body (2 Dutch types, 3 Estonian types). To assess differences in beta diversity across types of water body for each habitat use category, cumulative species richness curves were constructed, using BioDiversityProfessional Beta 1 (McAleece 1997). Cumulative species richness curves were based on averages of 250 random sorts of the sampling sites.

Table 2. Criteria for the classification of species in habitat use categories based on their abundance in stagnant water bodies in the ombrotrophic bog (ombr.), water bodies in the transitional mire and those influenced by flowing water (trans.), and in the lagg zone of the pristine raised bog system. # = number of individuals per species collected within each of these three parts, corrected for pond net sweep length and number of sampling sites.

<i>Habitat use category</i>	<i>Criteria</i>	<i>Number of species</i>
Ombrotrophic: stagnant water bodies in ombrotrophic bog massif	$\#_{\text{ombr}} > 2 * (\#_{\text{trans}} + \#_{\text{lagg}})$	48
Transitional: water bodies in transitional mire and ombrotrophic water bodies influenced by flow of acid water	$\#_{\text{trans}} > 2 * (\#_{\text{ombr}} + \#_{\text{lagg}})$	53
Lagg	$\#_{\text{lagg}} > 2 * (\#_{\text{trans}} + \#_{\text{ombr}})$	39
<i>If not classified in one of these three categories:</i>		
Ombrotrophic + Transitional	$(\#_{\text{ombr}} + \#_{\text{trans}}) > 0.75$	12
Transitional + lagg	$(\#_{\text{trans}} + \#_{\text{lagg}}) > 0.75$	3
<i>If not encountered in Estonian raised bogs:</i>		
Present in Estonia, but not encountered in sampling sites in raised bogs		40
Not present in Estonia		11

4.3 Results

Macroinvertebrate assemblage structure

The macroinvertebrate assemblages were clearly differentiated between the three Estonian and two Dutch types of water body (Figure 2). In Estonian raised bog landscapes the macroinvertebrate

species assemblage changed along a gradient in pH and calcium concentration, with the acid and large stagnant pools in the ombrotrophic bog massif on the one end and water bodies situated in the lagg on the other end. On this gradient, the species assemblage of the seven water bodies influenced by acid water flowing from a deeper peat layer, or by seasonal flow of acid water in depressions or at marginal slopes of the bog massif, was intermediate. The species assemblage varied considerably between the eight transitional mire sites, characterised by the presence of both *Sphagnum* mosses and vascular plant species characteristic for transitional mires; the assemblage in a primary bog lake and in deep open water pools fed by water from deeper peat layers resembled those in large stagnant ombrotrophic pools, whereas assemblages of water bodies in transitional mires varied, apparently depending on the permanence and amount of minerotrophic influence.

The Dutch remnant sites included the whole gradient from acid water bodies in the bog massif to the lagg of complete Estonian raised bog landscapes, although there were differences in species assemblage. Several remnant sites were inhabited by species assemblages that resembled those in temporary, more minerotrophic water bodies and sites with water flow in pristine bog landscapes. In the CA-plot restoration sites grouped closer together than the pristine and the remnants sites. Macroinvertebrate species composition in restoration sites showed some resemblance with only those in the acid part of the gradient in pristine bog landscapes, but clearly separated along the second CA-axis, and not to those characteristic of lags and transitional mires, except one restoration site previously influenced by inlet of surface water through a ditch. The species assemblage in deep ombrotrophic bog pools in Estonia differed from that in the large inundated industrial peat extraction sites and larger hand peat cuttings in The Netherlands, where chloride and nitrogen concentrations were higher (Figure 2).

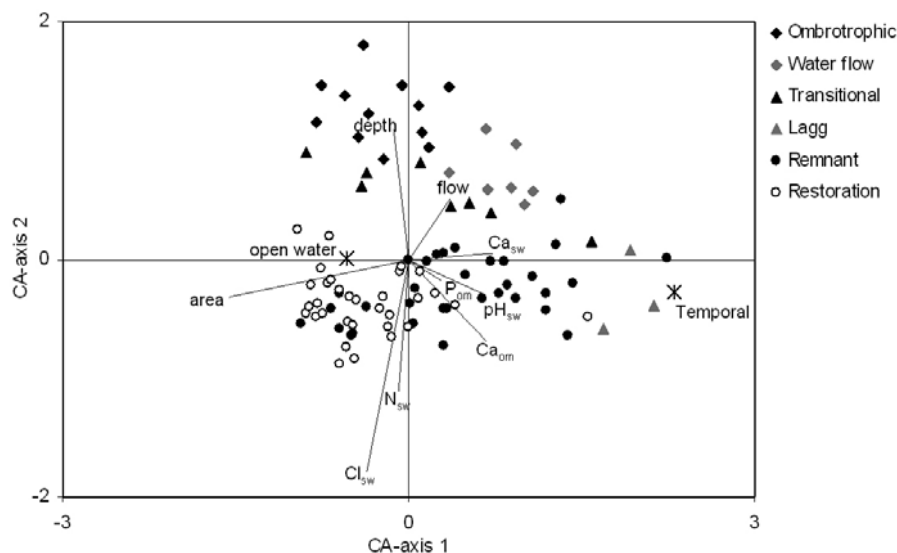


Figure 2. Plot of the Correspondence Analysis of log transformed species abundance data in Estonian bog landscapes (stagnant ombrotrophic bog pools, ombrotrophic water bodies influenced by flowing acid water at least part of the year, transitional mire, and lagg zone) and bog remnants in The Netherlands (remnant sites and restoration sites). Significant explaining variables are shown with lines for continuous variables (score multiplied by 5) and an asterisk for centroids of binomial variables (score multiplied by 3). sw=surface water; om=organic matter.

Ombrotrophic bog pools in Estonia and water bodies in Dutch bogs were inhabited by taxa of the same genus, but in each represented by different species. For example, the chironomid larva *Ablabesmyia phatta* dominated the macroinvertebrate assemblage in restoration sites (relative abundance 28%). In the pristine sites this species accounted for less than 1% of the total species abundance. Here, its congener *Ablabesmyia longistyla* was more abundant (relative abundance 6% in ombrotrophic sites), but this species was encountered infrequently and in low abundance in the Dutch sites. The chironomid larva *Psectrocladius* gr. *psilopterus* was the most abundant species in stagnant ombrotrophic pools, with a relative abundance of 16%, but accounted for less than 2% in the two groups of Dutch sites. Here, *Psectrocladius platypus* was the most abundant *Psectrocladius* species, like in the Estonian ombrotrophic water bodies with water flow and in transitional mires. In the ombrotrophic pools the dragonfly larva *Leucorrhinia dubia* was the most frequent *Leucorrhinia* species and had a similar relative abundance as *Leucorrhinia albifrons* (1%), whereas its congeneric species *Leucorrhinia rubicunda* was only recorded in transitional mires (Appendix). In The Netherlands, however, *L. rubicunda* was the most frequent and abundant *Leucorrhinia* species found in both remnant and restoration sites, whereas *L. dubia* was fairly rare and *L. albifrons* was absent. *Cymatia coleoptrata* was the most abundant aquatic heteropteran in both remnant and restoration sites and accounted for 2% of the macroinvertebrate abundance. This species was not encountered in pristine bogs. It is present in Estonia e.g. in minerotrophic lakes adjacent to a raised bog (unpublished data Bargerveen Foundation). In Estonian raised bogs its congener *Cymatia bonsdorffi* is the most abundant and frequent heteropteran species.

Species richness and abundance

In total 155 macroinvertebrate species were recorded in the 31 Estonian sites. Of these, 84 species were recorded in the 13 ombrotrophic sites, 112 in the 15 sites with water flow and in the transitional mire, and 53 in the three lagg sites. In total 164 species were recorded in the 65 Dutch sites. Of these, 147 species were recorded in the 33 remnant sites, and 116 species in the 32 restoration sites (Table 3 and Appendix). The geographic range of only 15 species did not include both Estonia and The Netherlands. Four species encountered in the Estonian sites were not native to The Netherlands and 11 species encountered in the Dutch sites were not native to Estonia. In total 46 species were found in the Estonian sites, but not in the Dutch sites, although native to The Netherlands. Almost half of these, 21 species, were very rare to fairly rare in The Netherlands according to Nijboer & Verdonschot (2001), including five dragonfly species listed in the Dutch Red Data Book (Wasscher et al. 1998). In total 40 species encountered in the Dutch sites were not encountered in the Estonian bog water bodies, although native to Estonia. Of these species, several species were highly frequent and abundant (e.g. *Cymatia coleoptrata*, *Endochironomus tendens*, *Glyptotendipes paripes*), suggesting invasion of degraded bogs by these species.

The average species richness and abundance did not differ significantly between the three types of water body in Estonian bogs, but there was clear differentiation between the habitat use categories (Figure 3). The average species richness in the Dutch remnant and restoration sites did not differ significantly from the Estonian sites. However, the total species abundance was significantly higher in the remnant and restoration sites than in the pristine ombrotrophic water bodies and in the transitional mire (Figure 2; ANOVA: $P < 0.001$). The abundances in Dutch sites were almost twice as high as in Estonian ombrotrophic and transitional mire water bodies.

Chapter 4

Table 3. Total number of species per habitat use category (see Table 2 for criteria) in the three distinguished types of water body in raised bog landscapes in Estonia and in the remnant and restoration sites in bog remnants in The Netherlands. Numbers of species between brackets refer to the species classified in their habitat use category based on occurrence in qualitative samples additional to that used for assessment of species abundance composition in Estonia. n= number of sampling sites.

Habitat use category	Estonia				The Netherlands			Total
	ombrotrophic (stagnant) n=13	water flow + transitional mire n=15	lagg n=3	total n=31	remnant n=33	restoration n=32	total n=65	
Ombrotrophic	48	25	3	48	31(+1)	31	34(+1)	48(+1)
Transitional	12	53	3	53	34(+3)	28(+3)	37(+4)	53(+4)
Lagg	9	19	39	39	22	13	22	39
Ombrotrophic + Transitional	12	12	5	12	9(+2)	9(+1)	10(+2)	12(+2)
Transitional + Lagg	3	3	3	3	2(+1)	2	2(+1)	3(+1)
Not in Estonian bogs	-	-	-	-	32	22	40	40
Not present in Estonia	-	-	-	-	10	7	11	11
Not present in The Netherlands	0	4	0	4	-	-	-	4
Total number of species	84	112	53	155	147	116	164	214

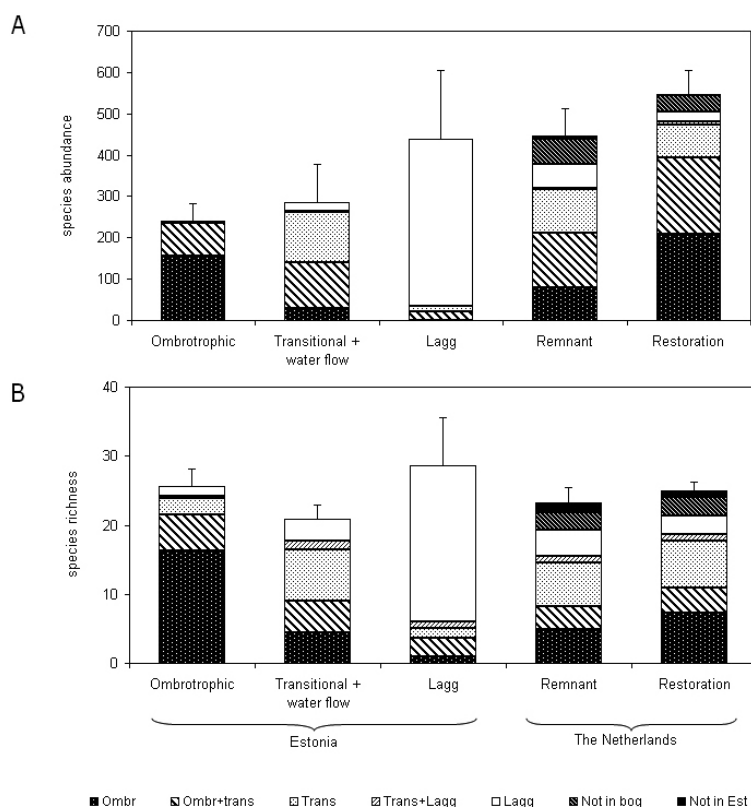


Figure 3. A) Average abundance of aquatic macroinvertebrate species per metre pond net sample (+SE) and B) average species richness per sampling site (+SE) in ombrotrophic sites, transitional mire sites and those influenced by flowing acid water, and lags in pristine raised bogs in Estonia and water bodies in remnant sites and restoration sites in The Netherlands. Species are classified according to their habitat use within pristine Estonian bog landscapes (see Table 2 for criteria).

Compared to the stagnant ombrotrophic bog pools in Estonia, the higher abundances in both groups of Dutch sites were primarily caused by species that were absent from Estonian bogs and species of the habitat use categories of transitional mires and laggs (Figure 3). Although most Dutch remnant sites were situated in former ombrotrophic parts of bog massifs, abundance of species from the stagnant ombrotrophic bog pools (habitat use category Ombr.) was low compared to the pristine stagnant ombrotrophic water bodies. In the restoration sites, created by retention of rain water, the species of this habitat use category showed a slightly increased abundance compared to pristine stagnant ombrotrophic water bodies. However, a lower average and cumulative number of species of this habitat use category together accounted for a higher abundance in the restoration sites than in the pristine stagnant ombrotrophic pools (Figure 3b; Figure 4a).

The habitat use category of both ombrotrophic and transitional mire sites (Ombr. + trans.) showed a higher abundance in both restoration and remnant sites compared to both the pristine stagnant ombrotrophic pools and the sites in the transitional mires and with water flow (Figure 3a). Again, the two groups of Dutch sites showed a lower average and cumulative species richness (Figure 3b and 4b, respectively).

The species of the three habitat use categories of transitional mires and laggs were slightly more abundant in remnant sites and scarcer in restoration sites, relative to pristine sites in transitional mires and with water flow (Figure 3a). The average species richness was similar in these three categories of sites (Figure 3b), but cumulative species richness was lower in remnant sites and lowest in restoration sites (Figure 4c).

4.4 Discussion

Aquatic macroinvertebrate assemblages differed in species abundance and species composition between water bodies in pristine Estonian raised bog landscapes and in Dutch bog remnants. The differences provide support for our first hypothesis that increased nutrient availability enabled certain macroinvertebrate species to invade bog massifs becoming abundant in nutrient enriched raised bog remnants. In addition, macroinvertebrate species composition differed between remnant and restoration sites, providing support for our second hypothesis that those macroinvertebrate species assemblages characteristic of laggs and transitional mires, including endangered species, hardly profit from the restoration practice focusing on ombrotrophic conditions solely.

Comparative study

Present-day Estonian raised bog landscapes remain marginally affected by human activities, like drainage and atmospheric pollution, making these potentially suitable references. Our assumption that macroinvertebrate assemblages in present-day Estonian raised bog landscapes resembled those of Dutch bogs in the past seems further justified as only 15 of the 214 species found in our study had a distribution area that did not encompass both countries, according to Fauna Europaea (2011) and own data. These species were not included in the correspondence analysis. Removing these species from our analysis is justified as they included only 1% of all macroinvertebrates collected in The Netherlands and 0.1% of all macroinvertebrates collected in Estonia. The Estonian bogs in our study were situated six degrees of latitude further to the North and have a slightly more continental climate compared to the Dutch sites. Mean January temperature is about 6 degrees Celsius lower in Western-Estonia, where most sampling sites

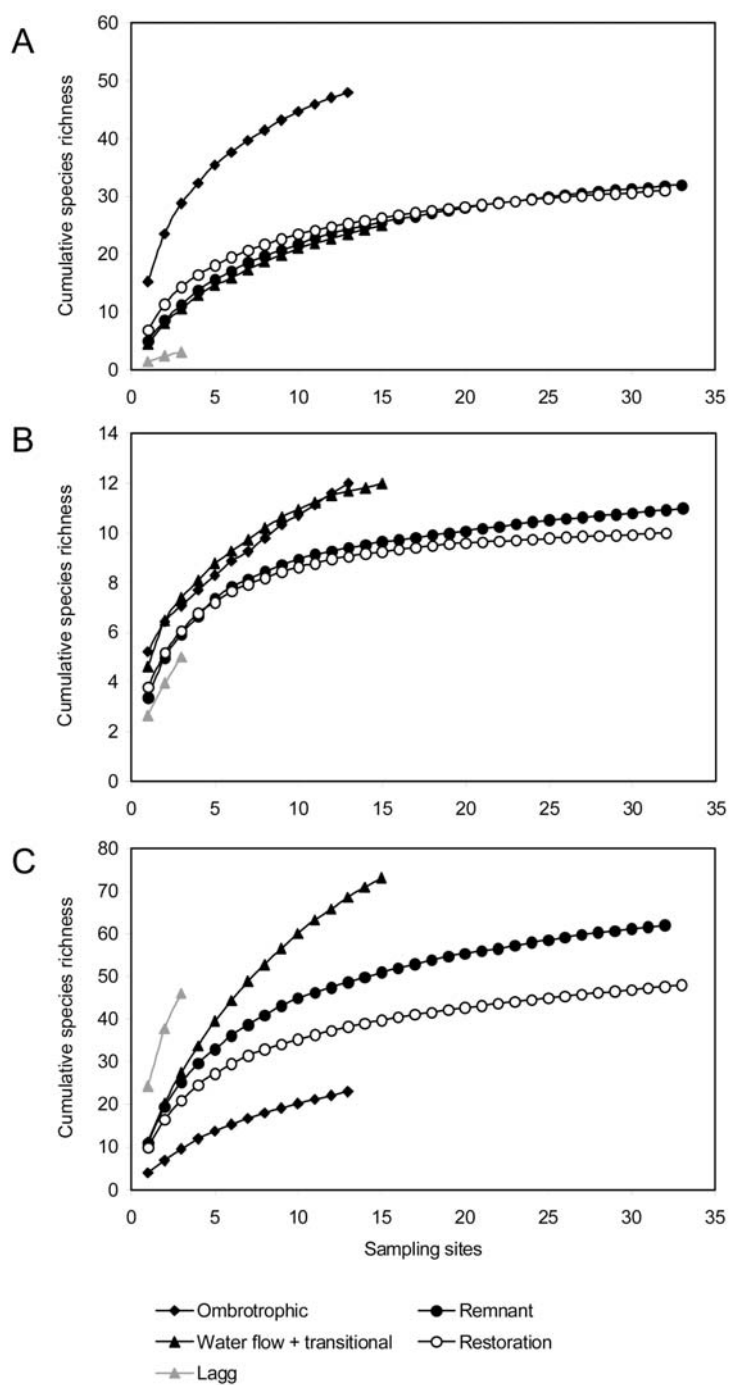


Figure 4. Cumulative species richness curves for the three types of water body in Estonian bog landscapes and remnant and restoration sites in The Netherlands. Curves for the species of the habitat use category of stagnant ombrotrophic pools (A), the habitat use category of ombrotrophic and transitional mire sites (B), and the three habitat use categories of transitional mires and lags together (C; see Table 2 for criteria). Note the different scales of the y-axes.

were situated, but mean temperature in summer is equal to The Netherlands (Estonian Meteorological and Hydrological Institute 2012, Royal Netherlands Meteorological Institute 2012). In the southern range of their distribution area, raised bogs are known for their cold climate relative to other habitat types and serve as refugia for 'glacial relict species' (Spitzer & Danks 2006). Hence, species restricted to bogs in the South may (also) occupy other types of habitat further North. Thus at least some of the 40 species that were only found in Dutch sites, although their distribution area encompassed both countries, could have already been present in pristine bogs in The Netherlands in the past. We cannot discount such shifts in habitat use of species across their geographical range, but note that if these shifts predominated, they would only obscure patterns in the abundance of different categories of habitat use. Yet, clear differences were found in abundance among habitat use categories and congeneric species, which clearly point to the effects of nutrient enrichment and loss of minerotrophic parts of bog landscapes (transitional mires and lags) in Dutch raised bog remnants.

Effects of nutrient enrichment

The differences between Estonian and Dutch sites in macroinvertebrate species assemblage and in abundance among habitat use categories and among congeners indicate that increased nutrient availability enables some species to increase, while constraining other species. Water bodies in raised bog remnants in The Netherlands show a dramatically increased availability of nitrogen and phosphate, compared to pristine raised bogs in Estonia (Table 1). In The Netherlands total macroinvertebrate abundance was found to be about two times higher compared to the most nutrient poor water bodies in the ombrotrophic bog massif in Estonia (Figure 3a), which would also suggest an increased food availability at the base of the foodchain. In terms of species composition, the increased abundances were primarily due to species that are naturally absent from pristine bogs or prefer sites in lags, transitional mires, or sites with water flow in the ombrotrophic bog massif. The habitat use of these species in the Estonian bog landscape suggests a dependency on locations with a higher productivity via increased availability of nutrients, higher decomposition rates, or both. This is further supported by the average preference for eutrophic and meso-eutrophic water bodies this group of 40 species, which are naturally absent from pristine bogs, shows in The Netherlands according to the species preferences assessed by Verberk et al. (2012). These species may thus have been facilitated in Dutch bog remnants where nutrient availability has been increased due to atmospheric deposition or mobilisation of nutrients after rewetting of drained and partly mineralised peat (Van Duinen et al. 2006a; Table 1).

The invasion of degraded bog massifs by species that do not naturally occur in pristine ombrotrophic conditions has also been found for different groups of aquatic and terrestrial macroinvertebrates in England, Finland, and Germany (Koponen 1979, Burmeister 1990, Andreessen 1993, Wheeler & Shaw 1995). This invasion of water bodies in Dutch bog remnants may have been at the expense of those species that are naturally restricted to the ombrotrophic bog pools, as indicated by the lower average and cumulative species richness of this habitat use category in The Netherlands (Figure 3b and 4a). Species preferring ombrotrophic pools may be superior competitors for food that is poor in quality, but lose their competitive edge when nutrient availability increases (sensu Tessier & Woodruff 2002, Iwabuchi & Urabe 2012). Species may be negatively affected by excess dietary nutrient content (Nijssen & Siepel 2010, Elser et al. 2012). Other mechanisms may also be involved and their elucidation requires further study of species traits and species responses to changes in habitat conditions. For instance, the chironomids *Lauterborniella grayloides* and *Pagastiella orophila*, present in ombrotrophic water bodies

in Estonian bogs and absent in Dutch bog remnants, also disappeared from Dutch moorland pools. This disappearance was attributed to acidification (Klink 1986). Their presence in several acid ombrotrophic pools in Estonia (see Appendix) indicates that they can tolerate high acidity, making oxygen shortage (Moller Pillot 2009) in these nutrient enriched water bodies, changes in food quality, or increased aluminium, ammonium or sulphide toxicity more likely mechanisms.

Effects of degradation and rewetting

The macroinvertebrate species composition in remnant sites included much more variation than that of restoration sites, as was apparent from the variation in species assemblage (Figure 2) and beta diversity (Figure 4). This has been previously reported for both spatial comparisons between restoration and remnant sites (Van Duinen et al. 2003) and temporal comparisons of sites before and after rewetting (Verberk et al. 2010a). The comparison with the species assemblages found in pristine Estonian raised bog landscapes revealed that assemblages in remnant sites largely overlapped with the whole gradient from acid water bodies in the bog massif to the lags of complete Estonian raised bog landscapes. Minerotrophic parts of pristine bog landscapes had their counterpart in temporary water bodies in Dutch bog remnants (Figure 2). Macroinvertebrate species assemblages of remnant sites include rare and characteristic species (Van Duinen et al. 2003) which have apparently been able to persist in these remnant sites during the gradual process of degradation of raised bog landscapes in the past centuries. Next to the gradual loss of suitable natural pools and puddles due to peat cutting and cultivation, man-made peat cutting pits and trenches apparently provided new habitat enabling these species to survive as relict populations. Restoration sites were limited to the acid part of the gradient, but the macroinvertebrate species composition was markedly different due to increased nutrient availability compared to pristine acid bog pools (see above). This difference was related to a large part to differences in species and not genera, showing that information at the level of species has much more discriminatory power than information at the genus level.

As reclamation started at the margins of bog landscapes, current bog remnants are in most cases parts of the former ombrotrophic bog massif (Van den Munckhof 1993, Wheeler & Shaw 1995). Consequently, transitional mires and lags have become rare and severely degraded. Groundwater influence is also lacking in most of these remnants. Consequently, several macroinvertebrate species that in Estonian raised bog landscapes prefer transitional mires and lags are absent or rare in Dutch bogs. Van Kleef et al. (2012) found that 21 out of a total of 33 endangered species (including vascular plant, odonates, water beetles and caddis flies) were recorded more often in the transitional mire and lagg zone of Estonian bogs than in the ombrotrophic bog massif. Apparently, these 21 species, as well as endangered species of taxonomic groups for which no Red List exists, do not profit from increased nutrient availability in Dutch bog remnants. This response is opposite to that for the species discussed above which also prefer transitional mires and lags of Estonian bogs but that have an increased abundance in Dutch bog remnants. This differential response of species indicates the aquatic macroinvertebrate communities of pristine transitional mires and lags to consist of species that have increased in The Netherlands and require a higher nutrient availability (Hypothesis 1) and species that have decreased in The Netherlands which are restricted to water bodies with a higher availability of minerals or acid buffering provided by the buffered ground water (Hypothesis 2), irrespective of a higher nutrient availability. A similar dichotomy was found for aquatic oligochaete species where this differential response could be linked to differences in their diet (Van Duinen et al. 2006a).

The lower cumulative species richness in restoration sites compared to pristine and remnant sites

(Figure 4) may also result from species' inability to recolonise rewetted bog sites (Van Duinen et al. 2006b, Van Duinen et al. 2007). Several restoration sites were created by rewetting of remnants of industrial peat extraction at locations where hardly any aquatic macroinvertebrates species could live prior to the rewetting measures. Development of species assemblages at these inundated sites should be judged as a positive result of these measures, even though the assemblage is not similar to those in pristine raised bogs. The average abundance and species richness of macroinvertebrate species preferring ombrotrophic pools was increased in restoration sites compared to remnant sites (Figure 3). This could also be judged as a positive result of restoration measures aiming at ombrotrophic conditions. Other studies showed the rapid colonisation and recovery of testate amoebae fauna or flies (Brachycera) after recovery of a *Sphagnum*-dominated vegetation on cut-over bogs (Buttler et al. 1996, Taillefer & Wheeler 2012) and aquatic macroinvertebrates after digging of pools in a cut-over bog (Mazerolle et al. 2006).

Implications for policy and restoration management

High nutrient availability limits the rehabilitation of aquatic macroinvertebrate communities of raised bog landscapes, like it hampers recovery of characteristic raised bog vegetation (Limpens et al. 2003, Tomassen et al. 2004). Therefore, a further reduction of atmospheric nitrogen deposition is necessary, while also measures to reduce the elevated phosphate concentration in Dutch bog remnants are required.

This study showed that aquatic macroinvertebrate species differ in their use of the different parts of the natural gradients between the ombrotrophic bog massif and adjacent minerotrophic habitat (Figure 2; Table 3). The loss of these gradients and the species dependent on these gradients calls for attention to optimization of the hydrological system up to catchment scale and conservation and restoration of heterogeneity or gradients in environmental conditions typical for raised bog landscapes. This can be achieved by restoring a high groundwater table under bog remnants and in buffer zones adjacent to bog remnants and upward seepage if possible in appropriate sites within and adjacent to bog remnants (Holden et al. 2004, Howie & Tromp-Van Meerveld 2011). Restoration of transitional habitat types could be on original locations inside or outside bog remnants, but also at locations adjacent to present-day bog remnants. In addition, perspectives for further development of such gradients are present in fen reserves, where buffered groundwater or surface water and sites in succession towards bog vegetation are present. Some fen reserves, and moorland pools as well, currently include hotspots of species typical for bog gradients (Van Kleef et al. 2012).

Rewetting may result in temporal or long-term unsuitable habitat for characteristic, rare, and endangered species. Re-colonisation capacity of these species is limited due to habitat fragmentation and limited dispersal ability of species. Several species were able to persist as relict population during the process of degradation in bog remnants. Our results confirm that not only degradation, but also restoration measures can act as a species filter, as was found earlier by Van Kleef et al. (2006) and Verberk et al. (2010a). Rewetting enables several species to increase their frequency and abundance, but may disturb the occurrence of other species. In particular bog specialists may be more sensitive to such a disturbance than generalists (Verberk et al. 2010b). In regions where natural and near-natural bogs still cover larger areas, such as in parts of Canada, Northern Europe, and Russia, chances of recolonisation may be higher than in The Netherlands, Germany and the UK, where raised bog remnants are surrounded by an extensive reclaimed area. Further studies are necessary to assess if this is also true for

species which have low dispersal abilities (Mazerolle & Poulin 2007, Van Duinen et al. 2007). However, the need for restoration of degraded bog remnants may be higher in more densely populated areas where natural and near-natural bogs are absent. In areas with low chances of recolonisation, persistence of species is an important aspect in the effectiveness of restoration measures. Therefore, preservation of relict populations of characteristic species in current bog remnants, fen reserves and in moorland pools is important.

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Chapter 4

Appendix

Species frequency in ombrotrophic water bodies, ombrotrophic water bodies with water flow and water bodies in transitional mires, and in lags in pristine Estonian raised bog landscapes and in remnant and restoration sites in The Netherlands. The habitat use categories in which species were classified based on their abundance within the different parts of Estonian bog landscapes is presented: ombr=ombrotrophic water bodies with stagnant water; trans=transitional mires and ombrotrophic water bodies with water flow (see Table 2 for criteria).

Species	Habitat use category	Estonia			The Netherlands	
		ombrotrophic n=13	water flow+ transitional		remnant n=33	restoration n=32
			mire n=15	lagg n=3		
Oligochaeta						
<i>Cernosvitoviella</i> spec.	lagg			0.67	0.03	
<i>Chaetogaster diaphanus</i>	not in bog				0.03	
<i>Cognettia glandulosa</i>	lagg		0.13	1.00	0.15	
<i>Cognettia sphagnetorum</i>	ombr+trans	0.92	0.87	0.67	0.27	0.41
<i>Dero digitata</i>	not in bog				0.06	0.03
<i>Limnodrilus udekemianus</i>	lagg			0.33		
<i>Lumbriculus variegatus</i>	lagg		0.47	1.00	0.27	0.25
<i>Mesenchytraeus</i> spec.	not in bog				0.03	
<i>Nais variabilis</i>	trans		0.27	0.67	0.36	0.47
<i>Pristina aequiseta</i>	trans		0.07		0.03	0.06
<i>Rhyacodrilus coccineus</i>	lagg			1.00		
<i>Rhynchelmis tetratheca</i>	lagg			1.00		
<i>Spirosperma ferox</i>	lagg			0.33		
<i>Stylodrilus brachystylus</i>	lagg			0.33		
<i>Stylodrilus heringianus</i>	not in bog				0.03	
<i>Tatriella slovenica</i>	lagg		0.27	0.67		
<i>Tubifex ignotus</i>	lagg			0.67		
<i>Vejdovskyella comata</i>	trans		0.07		0.03	
Odonata						
<i>Aeshna grandis</i>	ombr	0.08				
<i>Aeshna juncea</i>	ombr+trans	0.23	0.13			0.09
<i>Aeshna subarctica</i>	ombr	0.62	0.20			
<i>Anax imperator</i>	ombr	0.23				
<i>Ceragrion tenellum</i>	not in Est	-	-	-	0.12	0.19
<i>Coenagrion hastulatum</i>	trans		0.13			
<i>Coenagrion johanssoni</i> ²	trans		0.07		-	-
<i>Coenagrion lunulatum</i>	trans		0.07		0.15	0.47
<i>Coenagrion pulchellum/puella</i>	trans		0.13		0.18	0.16
<i>Cordulia aenea</i>	ombr	0.46	0.20		0.03	0.06
<i>Enallagma cyathigerum</i>	ombr	0.54	0.13		0.21	0.56
<i>Ischnura elegans</i>	not in bog				0.03	0.03
<i>Lestes sponsa/dryas</i>	ombr	0.54	0.13		0.03	0.03
<i>Lestes viridis</i>	not in Est	-	-	-		0.03
<i>Leucorrhinia albifrons</i>	ombr	0.54	0.07			
<i>Leucorrhinia dubia</i>	ombr	0.77	0.13		0.06	0.06
<i>Leucorrhinia rubicunda</i>	trans		0.20		0.39	0.53
<i>Libellula quadrimaculata</i>	ombr	0.38	0.20		0.27	0.34
<i>Pyrrhosoma nymphula</i>	trans		0.07		0.15	0.31
<i>Somatochlora arctica</i>	trans+lagg	0.08	0.40	0.33		

Restoration constraints for aquatic macroinvertebrates

Species	Habitat use category	Estonia			The Netherlands	
		water flow+ transitional			remnant	restoration
		ombrotrophic n=13	mire n=15	lagg n=3		
<i>Somatochlora flavomaculata</i>	lagg		0.07	0.33		
<i>Somatochlora metallica</i>	ombr	0.08	0.07		0.03	
Hemiptera						
<i>Callicorixa praeusta</i>	trans ¹					0.44
<i>Callicorixa wollastoni</i> ²	trans	0.15	0.27		-	-
<i>Corixa dentipes</i>	trans	0.15	0.07			
<i>Corixa punctata</i>	not in Est	-	-	-	0.18	0.31
<i>Cymatia bonsdorffi</i>	ombr	0.62	0.13		0.12	0.41
<i>Cymatia coleoptrata</i>	not in bog				0.24	0.69
<i>Gerris argentatus</i>	not in bog				0.03	
<i>Gerris gibbifer</i>	not in Est	-	-	-	0.03	
<i>Gerris lacustris</i>	lagg		0.13	0.33	0.03	0.03
<i>Gerris lateralis</i>	lagg			0.33		
<i>Gerris odontogaster</i>	ombr+trans ¹				0.15	0.19
<i>Glaenocoris propinqua</i>	ombr	0.08			0.03	0.09
<i>Hebrus pusillus</i>	not in bog				0.06	0.03
<i>Hebrus ruficeps</i>	lagg	0.08	0.20	0.33	0.21	0.16
<i>Hesperocoris castanea</i>	not in Est	-	-	-	0.15	
<i>Hesperocoris linnaei</i>	trans		0.07		0.06	0.03
<i>Hesperocoris sahlbergi</i>	trans+lagg	0.08	0.33	0.33	0.48	0.53
<i>Ilyocoris cimicoides</i>	ombr	0.62	0.07		0.18	0.19
<i>Limnoporus rufoscutellatus</i>	trans		0.07		0.03	
<i>Microvelia buenoi</i>	not in bog				0.06	0.09
<i>Microvelia reticulata</i>	ombr	0.15	0.07		0.39	0.53
<i>Nepa cinerea</i>	trans		0.07		0.06	0.03
<i>Notonecta glauca</i>	ombr	0.15	0.07		0.18	0.31
<i>Notonecta lutea</i>	ombr ¹				0.03	
<i>Notonecta obliqua</i>	not in Est	-	-	-	0.15	0.13
<i>Notonecta viridis</i>	not in Est	-	-	-	0.03	0.03
<i>Plea minutissima</i>	not in bog				0.09	
<i>Sigara distincta</i>	trans		0.07		0.06	
<i>Sigara falleni</i>	not in bog				0.03	
<i>Sigara limitata</i>	not in bog				0.03	0.09
<i>Sigara scotti/fossarum</i>	ombr	0.23			0.21	0.56
<i>Sigara semistriata</i>	trans	0.08	0.07			
<i>Sigara striata</i>	ombr	0.08			0.03	0.19
Chaoboridae						
<i>Chaoborus crystallinus</i>	ombr	0.08			0.39	0.25
<i>Chaoborus flavicans</i>	ombr	0.08			0.09	0.06
<i>Chaoborus obscuripes</i>	ombr	0.38			0.18	0.19
<i>Chaoborus pallidus</i>	trans		0.07		0.27	0.25
<i>Mochlonyx spec.</i>	trans		0.20		0.45	0.09
Dixidae						
<i>Dixella aestivalis</i>	ombr+trans	0.08	0.07		0.03	0.03
<i>Dixella amphibia</i>	lagg		0.13	0.67	0.06	0.03
Chironomidae						
<i>Ablabesmyia longistyla</i>	ombr	0.77	0.33	0.33	0.06	0.06
<i>Ablabesmyia monilis</i> agg.	ombr	0.15				

Chapter 4

Species	Habitat use category	Estonia			The Netherlands	
		water flow+ transitional			remnant	restoration
		ombrotrophic n=13	mire n=15	lagg n=3		
<i>Ablabesmyia phatta</i>	ombr	0.62	0.13		0.33	0.72
<i>Ablabesmyia</i> spec.	ombr	0.85	0.40		0.09	0.16
<i>Acricotopus lucens</i>	not in bog				0.03	
<i>Chaetocladius</i> spec.						
Herkenbosch	trans	0.15	0.33		0.03	0.03
<i>Chironomus</i> spec.	ombr+trans	0.77	0.73	1.00	0.76	1.00
<i>Conchapelopia</i> spec.	trans		0.07			
<i>Corynoneura</i> spec.	trans	0.38	0.47	0.33	0.27	0.34
<i>Cricotopus</i> gr. <i>sylvestris</i>	not in bog					0.03
<i>Dicrotendipes</i> spec.	ombr	0.31	0.07		0.03	
<i>Endochironomus albipennis</i>	trans		0.07			0.09
<i>Endochironomus</i> gr. <i>dispar</i>	lagg	0.15	0.07	0.67	0.21	0.34
<i>Endochironomus tendens</i>	not in bog				0.27	0.47
<i>Glyptotendipes pallens</i> agg.	trans		0.07			
<i>Glyptotendipes paripes</i>	not in bog				0.21	0.69
<i>Guttipielopia guttipennis</i>	not in bog				0.06	0.03
<i>Lasiodiamesa</i> spec.	lagg	0.08	0.33	1.00	0.12	
<i>Lauterborniella agrayloides</i>	ombr	0.23				
<i>Limnophyes</i> spec.	lagg	0.08	0.13	0.33	0.39	0.22
<i>Macropelopia</i> spec.	trans		0.40		0.12	
<i>Metriocnemis</i> spec.	trans		0.07			0.03
<i>Micropsectra</i> spec.	trans		0.07	0.33	0.06	0.03
<i>Microtendipes chloris</i> agg.	ombr	0.15	0.07			
<i>Monopelopia tenuicalcar</i>	ombr	1.00	0.47	0.33	0.70	0.63
<i>Natarsia</i> spec.	lagg		0.20	0.67	0.06	0.13
<i>Pagastiella orophila</i>	ombr	0.15				
<i>Parachironomus</i> gr. <i>arcuatus</i>	trans		0.07		0.03	
<i>Paralimnophyes hydrophilus</i>	lagg			0.33	0.18	0.22
<i>Paratanytarsus</i> spec.	ombr	0.08				0.03
<i>Paratendipes nudisquama</i>	ombr	0.62	0.40		0.15	0.16
<i>Polypedilum sordens</i>	trans	0.08	0.07		0.06	0.06
<i>Polypedilum uncinatum</i> agg.	lagg	0.38	0.20	1.00	0.55	0.59
<i>Procladius</i> s.l.	trans	0.62	0.67		0.64	0.91
<i>Psectrocladius</i> gr. <i>psilopterus</i>	ombr	0.77	0.53	0.33	0.39	0.44
<i>Psectrocladius</i> gr.						
<i>sordidellus/limbatellus</i>	ombr+trans	1.00	0.60	0.33	0.27	0.66
<i>Psectrocladius platypus</i>	ombr+trans	0.92	0.87	0.33	0.79	0.69
<i>Psectrotanytus varius</i>	not in bog				0.03	0.06
<i>Pseudochironomus</i> spec.	trans		0.07			
<i>Pseudosmittia</i> spec.	ombr	0.08				
<i>Stenochironomus</i> spec.	not in bog				0.06	
<i>Tanytarsus</i> spec.	trans	0.31	0.27		0.24	
<i>Telmatopelopia nemorum</i>	lagg		0.07	0.67	0.24	0.28
<i>Trissocladius</i> spec.	lagg			0.33		
<i>Xenopelopia</i> spec.	not in Est	-	-	-	0.12	
<i>Zavrelimyia</i> spec.	lagg			0.33	0.03	
Coleoptera						
<i>Acilius canaliculatus</i>	ombr+trans	0.15	0.13		0.24	0.25
<i>Acilius sulcatus</i>	ombr	0.08			0.06	0.06

Restoration constraints for aquatic macroinvertebrates

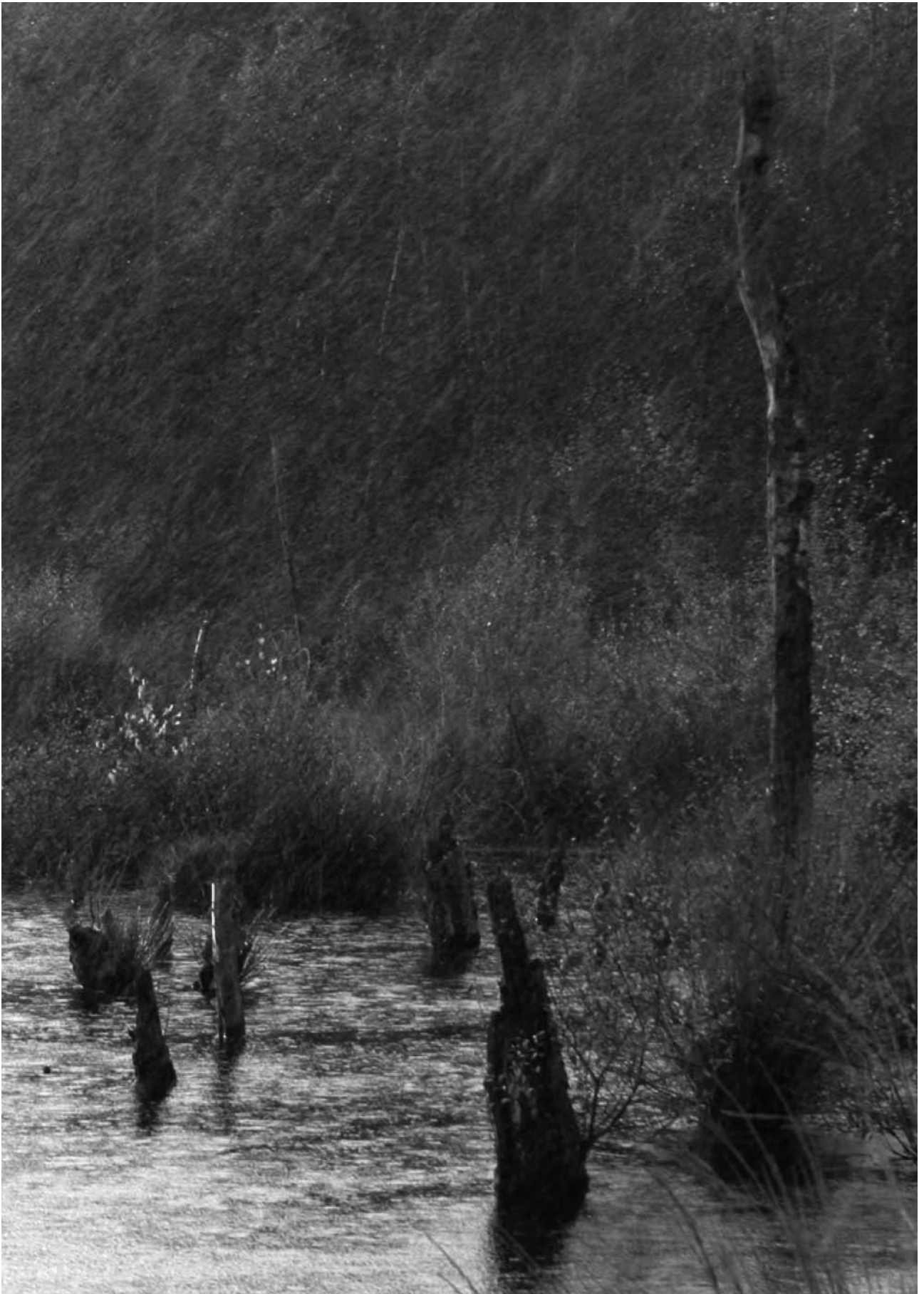
Species	Habitat use category	Estonia			The Netherlands	
		ombrotrophic n=13	water flow+ transitional mire n=15	lagg n=3	remnant n=33	restoration n=32
<i>Agabus bipustulatus</i>	trans		0.13		0.21	0.09
<i>Agabus congener</i>	trans+lagg ¹				0.09	
<i>Agabus labiatus</i>	not in bog				0.03	
<i>Agabus striolatus</i>	lagg			0.67		
<i>Agabus sturmii</i>	trans		0.07		0.03	0.13
<i>Anacaena lutescens</i>	lagg	0.08	0.07	0.33	0.33	0.06
<i>Berosus luridus</i>	not in bog					0.03
<i>Bidessus grossepunctatus</i>	ombr	0.08				
<i>Bidessus unistriatus</i>	ombr	0.62	0.07		0.09	0.03
<i>Cercyon convexiusculus</i>	not in bog					0.03
<i>Coelambus impressopunctatus</i>	not in bog				0.09	0.03
<i>Colymbetes fuscus</i>	not in bog				0.03	0.03
<i>Copelatus haemorrhoidalis</i>	not in bog				0.03	0.03
<i>Dytiscus lapponicus</i>	ombr	0.15				
<i>Dytiscus marginalis</i>	ombr+trans ¹				0.03	
<i>Enochrus affinis</i>	ombr+trans	0.08	0.13		0.36	0.13
<i>Enochrus coarctatus</i>	ombr	0.08			0.09	0.06
<i>Enochrus ochropterus</i>	ombr	0.15			0.15	0.25
<i>Graphoderus cinereus</i>	not in bog					0.03
<i>Graphoderus zonatus</i>	ombr	0.08			0.03	
<i>Haliphus spec.</i>	lagg			0.33	0.12	
<i>Helophorus punctatus</i>	not in Est	-	-	-	0.39	0.25
<i>Helophorus aequalis</i>	not in Est	-	-	-	0.27	
<i>Helophorus brevipalpis</i>	trans		0.07		0.18	
<i>Helophorus flavipes</i>	not in bog				0.06	
<i>Helophorus grandis</i>	lagg		0.07	0.33		
<i>Helophorus griseus</i>	not in bog				0.03	
<i>Helophorus laticollis</i>	trans		0.07			
<i>Helophorus minutus</i>	not in bog				0.03	
<i>Hydraena spec.</i>	lagg			0.33		
<i>Hydrobius fuscipes</i>	lagg	0.15	0.07	0.33	0.09	
<i>Hydrochus spec.</i>	lagg			0.67	0.06	
<i>Hydroglyphus pusillus</i>	ombr	0.62			0.12	0.03
<i>Hydroporus angustatus</i>	ombr	0.08				
<i>Hydroporus erythrocephalus</i>	trans	0.15	0.13		0.52	0.34
<i>Hydroporus gyllenhalii</i>	not in bog				0.27	
<i>Hydroporus incognitus</i>	trans		0.13		0.03	
<i>Hydroporus melanarius</i>	trans		0.13		0.12	
<i>Hydroporus morio</i>	trans		0.07			
<i>Hydroporus nigrita</i>	trans		0.13			
<i>Hydroporus obscurus</i>	ombr+trans	0.77	0.73	0.33	0.30	0.19
<i>Hydroporus palustris</i>	trans		0.20		0.06	0.06
<i>Hydroporus planus</i>	trans ¹				0.24	0.03
<i>Hydroporus pubescens</i>	not in bog				0.30	0.13
<i>Hydroporus scalesianus</i>	trans ¹				0.06	0.09
<i>Hydroporus striola</i>	trans		0.07			
<i>Hydroporus tristis</i>	lagg	0.23	0.33	0.67	0.55	0.19

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Species	Habitat use category	Estonia			The Netherlands	
		water flow+ transitional			remnant	restoration
		ombrotrophic n=13	mire n=15	lagg n=3	n=33	n=32
<i>Hydroporus umbrosus</i>	trans+lagg	0.23	0.47	0.33	0.30	0.38
<i>Hygrotus decoratus</i>	lagg			0.33	0.06	0.22
<i>Hygrotus inaequalis</i>	trans	0.08	0.13		0.33	0.56
<i>Hygrotus versicolor</i>	trans		0.07			
<i>Hyphydrus ovatus</i>	trans	0.15	0.20		0.03	0.13
<i>Ilybius aenescens</i>	trans		0.07			0.06
<i>Ilybius guttiger</i>	ombr+trans	0.08	0.13			
<i>Laccobius bipunctatus</i>	not in bog				0.03	
<i>Laccophilus minutus</i>	not in bog				0.03	
<i>Laccophilus poecilus</i>	ombr	0.31				
<i>Noterus crassicornis</i>	trans		0.13		0.36	0.25
<i>Rhantus grapii</i>	trans ¹				0.03	
<i>Rhantus exsoletus</i>	not in bog					0.03
<i>Rhantus suturalis</i>	not in bog				0.09	
<i>Rhantus suturellus</i>	ombr+trans	0.08	0.20		0.06	
Trichoptera						
<i>Agrypnia obsoleta</i>	ombr	0.23	0.07			0.09
<i>Agrypnia picta</i> ²	trans		0.07		-	-
<i>Agrypnia varia</i>	ombr	0.23			0.06	0.13
<i>Anabolia brevipennis</i>	lagg			0.33		
<i>Cyrtus flavidus</i>	ombr	0.31	0.07			0.03
<i>Ecnomus tenellus</i>	ombr	0.08	0.13			
<i>Glyptotendipes pellucidus</i>	lagg			1.00		
<i>Hagenella clathrata</i>	lagg		0.13	0.33	0.06	
<i>Holocentropus dubius</i>	ombr	0.77	0.20		0.12	0.50
<i>Holocentropus picicornis</i>	not in bog					0.03
<i>Holocentropus stagnalis</i>	trans	0.08	0.13		0.30	0.47
<i>Limnephilus elegans</i>	trans		0.07		0.03	0.03
<i>Limnephilus flavicornis</i>	not in bog					0.03
<i>Limnephilus luridus</i>	not in Est	-	-	-	0.03	0.03
<i>Limnephilus marmoratus</i>	not in bog				0.03	
<i>Limnephilus nigriceps</i>	lagg			0.33	0.03	
<i>Limnephilus politus</i>	not in bog				0.03	
<i>Limnephilus stigma</i>	lagg			1.00		
<i>Molannodes tinctus</i>	not in bog					0.03
<i>Oligostomis reticulata</i>	trans		0.13			
<i>Oligotricha lapponica</i> ²	trans		0.07		-	-
<i>Oligotricha striata</i>	trans		0.13		0.06	0.28
<i>Triaenodes bicolor</i>	ombr+trans	0.08	0.07			
<i>Trichostegia minor</i>	lagg	0.08	0.13	1.00		

¹) Habitat use category based on species occurrence in qualitative samples additional to that used for assessment of species abundance in Estonia.

²) Not native to The Netherlands.



Chapter 5

Differential response of aquatic oligochaete species to increased nutrient availability - a comparative study between Estonian and Dutch raised bogs

Gert-Jan van Duinen, Tarmo Timm, Fons Smolders, Ankie Brock, Wilco Verberk & Hans Esselink

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◀ Nutrient-poor ecosystems like raised bogs are especially sensitive to increased nutrient availability. The nutrient availability in this lagoon in the Mariapeel, The Netherlands, is relatively high due to increased peat decomposition caused by former drainage for peat extraction, inlet of surface water, pollution by sulphur compounds, as well as wet and dry deposition of atmospheric nitrogen.

Abstract

To assess the effects of increased nutrient availability on aquatic oligochaetes in raised bogs, species assemblages were compared within and between fairly pristine raised bogs in Estonia and raised bog remnants in The Netherlands. Within the pristine bog landscape a distinct pattern in the species assemblage is present. In the most nutrient-poor water bodies, in the ombrotrophic raised bog, only the fragmenting, almost never mature, acid-tolerant species *Cognettia sphagnetorum* is present. In pristine Estonian raised bogs *Nais variabilis*, *Lumbriculus variegatus* and sexually reproducing species are limited to more minerotrophic water bodies, which have a higher decomposition rate of dead organic matter and, consequently, higher nutrient availability. With ten species the lagg zone, is the most species-rich part of a pristine raised bog landscape. Most of these lagg zone species are not present in Dutch bog remnants as this part of the bog landscape has long been cultivated. *Nais variabilis* occurs in the Dutch bog remnants much more frequently than in Estonian bogs, whereas the frequency of *C. sphagnetorum* and *L. variegatus* is similar between both countries. These three species respond differently to the increased nutrient availability in The Netherlands, which could be linked to differences in their diets. In contrast to pristine bog pools, *N. variabilis* in Dutch raised bog remnants is present in water bodies not influenced by minerotrophic water. In Dutch raised bog remnants the occurrence of oligochaetes is not limited anymore by nutrient availability, due to the higher atmospheric nitrogen and sulphur loads in The Netherlands. Overall, it can be concluded that the degradation of Dutch raised bogs has resulted in the loss of both the nutrient-poor parts of the landscape and the special lagg conditions.

5.1 Introduction

Increase of nutrient availability causes shifts in aquatic and terrestrial plant species composition and productivity, and, consequently, may alter faunal species assemblages. Nutrient-poor ecosystems, like raised bogs, are especially sensitive to increased nutrient availability (e.g. Bobbink et al. 1998, Risager 1998). Most raised bogs remaining in north-western Europe are not only affected by drainage and peat cutting (Schouwenaars 1993), but also by increased deposition of atmospheric nitrogen (Berendse et al. 2001) and sulphur compounds (Roelofs 1986, Leuven 1988). Studies in lakes, rivers and ditches have shown that increased nutrient availability affects the aquatic invertebrate assemblages (e.g. Lang 1999, Lang & Reymond 1995, Verdonshot 1996). Compared to the extremely nutrient poor raised bogs, those aquatic systems have a relatively high nutrient availability under unpolluted conditions. Effects of increased nutrient availability on aquatic invertebrates in raised bogs, however, are barely studied.

Comparison of the species assemblages of coleopterans, heteropterans, trichopterans, and odonates between water bodies in the fairly pristine raised bog system Nigula (Estonia) showed that variation in the species assemblage is related to natural variation in the availability of minerals and nutrients (Smits et al. 2002), among other habitat characteristics. The species assemblage of the most nutrient poor, ombrotrophic pristine bog pools in Nigula is not found in raised bog remnants in The Netherlands (Van Duinen et al. 2002). The species assemblage of Dutch bog remnants was more similar to those of the Estonian water bodies with a naturally higher nutrient availability, such as transitional mires and bog brooklets. This pattern may well be attributed to the relatively high nutrient load in The Netherlands, lifting bottlenecks for the occurrence of species in ombrotrophic water bodies. However, the causal relations giving rise to these changes in species assemblages are not yet elucidated. The species studied by Van Duinen et al. (2002) included many carnivores, whose occurrence is probably related to increased

nutrient availability via the nutrient content and density of animals from a lower trophic level. The occurrence of herbivores and detritivores is expected to be more directly related to nutrient availability. This paper focuses on the aquatic oligochaetes of which many species feed either directly on dead organic matter, or on algae, fungi, and bacteria growing on organic matter (Learner et al. 1978).

In raised bogs the quality and decomposition rate of dead organic matter and the microbial community is altered by increased nitrogen and sulphur deposition. Increased N deposition results in a higher N availability in the surface water and interstitial water and in a higher N content of *Sphagnum* mosses (Lamers et al. 2000, Limpens et al. 2003a). Increased N availability also increases the cover of vascular plants such as *Molinia caerulea* and *Betula* spp. (Hogg et al. 1995, Lamers et al. 2000, Limpens et al. 2003a, Tomassen et al. 2003 and 2004b). Thus, increased N deposition results in an increased input of dead organic vascular plant material to the water bodies. As a consequence, the decomposition rate of dead organic matter might increase (Lamers et al. 2000), as nutrient availability and litter quality usually limit the activity of decomposing bacteria and fungi in pristine raised bogs (Belyea 1996, Smolders et al. 2002). Next to litter quality, a higher pH, buffering capacity, and SO_4 availability stimulate organic matter decomposition and N and P availability (Kok & Van de Laar 1991, Lamers et al. 1998 and 1999, Smolders et al. 2002, Tomassen et al. 2004a). SO_4 serves as a terminal electron acceptor for bacteria, thus increasing anaerobic oxidation of organic matter. This results in an increased decomposition rate of organic matter and an increased release of NH_4 and PO_4 in bog water bodies (Lamers et al. 1998). Growth of algae is increased by increased availability of N and P (Gulati & DeMott 1997, Limpens et al. 2003b).

Aquatic oligochaetes feeding on algae are expected to increase with increased N and P availability in the surface water and consequent increases in algal growth, whereas detritivorous oligochaetes -feeding on dead organic matter, fungi, and bacteria- are expected to increase with a higher nutrient content and decomposition rate of dead organic matter. Here we test the hypothesis that the aquatic oligochaete species whose occurrence in pristine Estonian bogs is limited to water bodies with a higher nutrient availability are in Dutch raised bog remnants not limited anymore by nutrient availability, as nutrient availability is high overall. To test this hypothesis, the occurrence of oligochaete species as well as nutrient availability in dead organic matter, interstitial water, and surface water were compared between 1.) water bodies naturally differing in nutrient availability within Estonian pristine raised bog landscapes and 2.) a variety of water bodies in Dutch raised bog remnants affected by increased N and S deposition.

In this paper we will answer the following questions:

1. What oligochaete species are present in pristine Estonian raised bogs and does their presence differ between the ombrotrophic raised bog, transitional mire and lagg zone?
2. Which of these oligochaete species are present in Dutch raised bog remnants?
3. Do the oligochaete species in raised bogs respond differently to nutrient availability in surface water or nutrient content and decomposition rate of dead organic matter?

5.2 Materials and methods

Study areas

In Estonia 27 water bodies were sampled in Nigula Nature Reserve: 20 in the ombrotrophic raised bog, six in the transitional mire, spring and connected pools, and one in the lagg zone. In

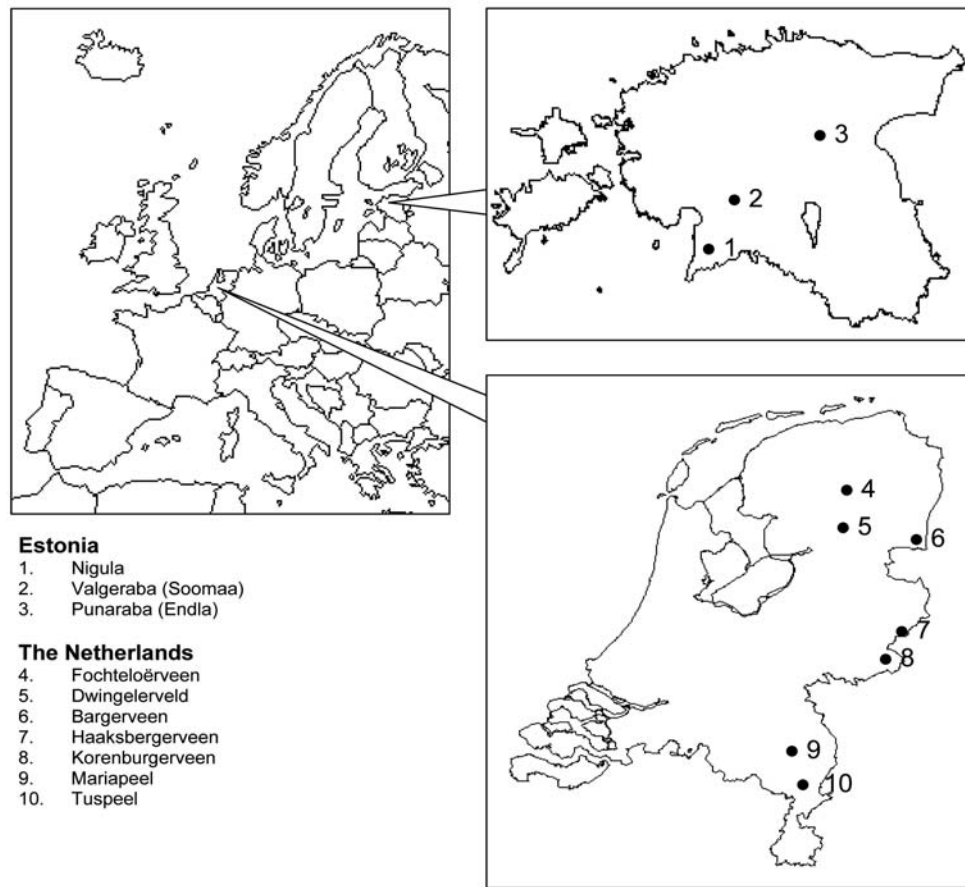


Figure 1. Location of study areas in Estonia and The Netherlands.

addition, two samples were taken in the transitional mire of Valgeraba, Soomaa National Park, and two samples in the lagg of Punaraba, Endla Nature Reserve (Figure 1). In The Netherlands samples were taken at 46 sites in seven raised bog remnants. Sampling sites included most of the various types of water body present in both pristine raised bogs (see Smits et al. 2002) and Dutch raised bog remnants (see Van Duinen et al. 2003). The water bodies sampled differed in size, water and organic matter quality, vegetation composition and structure. Despite the recent decrease in atmospheric N and S deposition in The Netherlands (RIVM 2004), deposition is still among the highest in western Europe (Jonson et al. 1998, Alcamo et al. 2002) and N deposition is considerably higher than at the study areas in Estonia (c.f. Pajuste et al. 2002, Tomassen et al. 2004b).

Oligochaete sampling

To sample most microhabitats present in the selected water bodies, a pond net (20x30 cm with 0.5 mm mesh size) was swept from the substrate and more or less open water into more dense vegetation near the shore over a distance of at least one metre. If dense vegetation was lacking, one or more longer sweeps were taken to collect at least 100 macroinvertebrates in a sample. In very dense *Sphagnum* vegetation, where invertebrate density is generally high, short sweeps (20

to 50 cm) were made to avoid clogging of the pond net. The Dutch sites were sampled in spring 1999. The Estonian sites were sampled in spring 2001. All samples were washed over three sieves with 2, 1, and 0.5 mm mesh sizes respectively and sorted in white trays. Oligochaetes were found in 38 of the Dutch sampling sites and in 22 of the Estonian sites. Although some oligochaetes might have been missed by the sampling method used in this study, the method did not selectively influence the species data of any category of sampling sites and did therefore not affect the findings of this study. Oligochaetes were stored in 4 % formalin, identified to species level if possible using the key of Timm (1999), and deposited in the corresponding authors collection.

Environmental variables

In Estonia dead organic matter (top layer of accumulated peat and sedimented detritus), interstitial water, and surface water were collected in May and June 2001. At the Dutch sampling sites surface water and interstitial water were sampled one to four times between October 1998 and October 1999. Dead organic matter was collected once in August 1999. In The Netherlands interstitial water was sampled using ceramic cups pressed into the top layer of the organic matter bottom. In Estonia Rhizon soil moisture samplers were pressed into the samples of the top layer of the organic matter. These samplers (both types produced by Eijkelkamp Agrisearch Equipment, Giesbeek, The Netherlands) were connected to vacuumed PVC syringes (50 ml).

The pH of surface water was measured in the field. The pH of water samples was measured within 24 hours. After 1 mg citric acid per 5 ml of water had been added, water samples were stored at -20°C until analysis. Organic matter was dried (48 hours at 70°C) and ground up in liquid nitrogen. Nitrogen and carbon concentrations were measured in dried samples with a CNS analyzer (type NA 1500; Carlo Erba Instruments, Milan, Italy). Digestates of ground-dried organic matter were prepared with the aid of a Milestone microwave (type mls 1200 Mega, Serisole, Italy), using nitric acid and hydrogen peroxide. Digestates were analyzed for total-P, Ca, and Mg. The analysis of the water samples included determination of the concentrations of NO₃, NH₄, o-PO₄ and S (for methods used see Van Duinen et al. 2003). The Ca + Mg content of organic matter was corrected for ash content.

Data analysis

Statistical significance of differences in environmental variables between the sampling sites in the ombrotrophic and minerotrophic parts of the Estonian raised bogs and between Estonian and Dutch sites was tested with an independent samples t-test. To assess the species' responses to water and organic matter variables linear regression analysis of log-transformed species abundance and chemical data from Estonian and Dutch sampling sites was performed using SPSS version 11.0 for Windows.

5.3 Results

Estonian raised bogs

In the Estonian raised bogs 13 oligochaete species were found (Table 1). The fragmenting enchytraeid *Cognettia sphagnetorum* was most frequently encountered and was the only species found in the water bodies of the ombrotrophic part of the raised bog landscape. This species was also present at various other sampling sites, always accompanied by other oligochaete species. In the transitional mires, spring, and connected pools assemblages of three to five oligochaete species were found. The highest number of species (11) was found in the lagg zone of Nigula bog.

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Table 1. Number of sites in which oligochaete species were encountered in the distinguished parts of the raised bog landscapes in Estonia and in the raised bog remnants in The Netherlands. n= total number of sampling sites. Ng= Nigula; Vr= Valgeraba; Pr= Punaraba. Dominant mode of reproduction according to Timm (1970): A= architomy (=fragmentation); P= paratomy (=budding); S= sexual.

		ombrotrophic	transitional mire/spring	lagg	Estonia	The Netherlands
Species	Reproduction	Ng n=20	Ng/Vr n=8	Ng/Pr n=3	total n=31	total n=46
Naididae						
<i>Nais variabilis</i> Piguët, 1906	P		4	1	5	20
<i>Pristina aequiseta</i> Bourne, 1891	P					1
<i>Dero digitata</i> (Müller, 1774)	P					3
Tubificidae						
<i>Rhyacodrilus coccineus</i> (Vejdovský, 1875)	S			2	2	
<i>Limnodrilus udekemianus</i> Claparède, 1862	S			1	1	
<i>Spirosperma ferox</i> Eisen, 1879	S			1	1	
<i>Tubifex ignotus</i> (Štolc, 1886)	S			1	1	
Tubificidae gen. sp. juv. (with hair chaetae)			3		3	2
Enchytraeidae						
<i>Cernosvitoviella</i> sp.	S			1	1	
<i>Cognettia sphagnetorum</i> (Vejdovský, 1878)	A	11	4	1	16	18
<i>Cognettia glandulosa</i> (Michaelsen, 1888)	A		2	2	4	
Lumbricidae						
<i>Lumbriculus variegatus</i> (Müller, 1774)	A		7	3	10	13
<i>Stylodrilus brachystylus</i> Hrabě, 1929	S			1	1	
<i>Stylodrilus heringianus</i> Claparède, 1862	S					1
<i>Tatriella slovenica</i> Hrabě, 1939	S		2	1	3	
<i>Rhynchelmis tetraethica</i> Michaelsen, 1920	S			2	2	
Total number of species		1	6	12	13	7

Table 2. Chemical data (means $\pm$ standard error) of the sampling sites in the distinguished parts of the raised bog landscapes in Estonia. Ng= Nigula; Vr= Valgeraba; Pr= Punaraba. Significance of the differences between the means of ombrotrophic pools and the other parts of the pristine bogs is indicated using the *P*-values of the t-test. n.s. = $P > 0.05$, * = $P < 0.05$, ** = $P < 0.01$, and *** = $P < 0.001$.

	ombrotrophic	transitional mire/spring	lagg	ombrotr. vs. other parts	
	Ng n=20	Ng/Vr n=8	Ng/Pr n=3	excl.lagg <i>P</i>	incl.lagg <i>P</i>
surface water					
pH	3.9 $\pm$ 0.04	4.5 $\pm$ 0.22	5.5 $\pm$ 0.50	*	**
<i>o</i> -PO ₄ (μmol l ⁻¹)	0.4 $\pm$ 0.03	0.8 $\pm$ 0.50	0.4 $\pm$ 0.15	n.s.	n.s.
NO ₃ + NH ₄ (μmol l ⁻¹)	6.0 $\pm$ 0.74	13 $\pm$ 6.1	3.7 $\pm$ 0.71	n.s.	n.s.
Ca + Mg (μmol l ⁻¹)	21 $\pm$ 2.5	68 $\pm$ 15	221 $\pm$ 30	**	**
interstitial water					
pH	4.5 $\pm$ 0.07	5.1 $\pm$ 0.23	5.8 $\pm$ 0.30	*	**
<i>o</i> -PO ₄ (μmol l ⁻¹)	0.2 $\pm$ 0.05	0.2 $\pm$ 0.04	4.3 $\pm$ 2.7	n.s.	n.s.
NO ₃ + NH ₄ (μmol l ⁻¹)	9.6 $\pm$ 3.4	8.2 $\pm$ 3.8	2.5 $\pm$ 0.2	n.s.	n.s.
Ca + Mg (μmol l ⁻¹)	25 $\pm$ 4.0	110 $\pm$ 28	312 $\pm$ 95	**	**
organic matter					
Ca + Mg (μmol g ⁻¹ DW)	81 $\pm$ 4.9	201 $\pm$ 41	561 $\pm$ 230	*	*
C:N quotient (g g ⁻¹)	30 $\pm$ 2.2	31 $\pm$ 4.8	18 $\pm$ 1.0	n.s.	n.s.
C:P quotient (g g ⁻¹)	1220 $\pm$ 105	698 $\pm$ 85	311 $\pm$ 84	***	***

Lumbriculus variegatus and *Nais variabilis* were the second and third most frequently occurring species. These three species most frequently reproduce asexually. Sexually reproducing species were only present in the lagg zones, apart from *Tatriella slovenica*, which was also found in the spring of the bog brooklet in Nigula bog and the transitional mire of Valgeraba. In this spring, very small and fragmenting (often exhibiting regeneration either on posterior or anterior ends)

Response of oligochaetes to increased nutrient availability

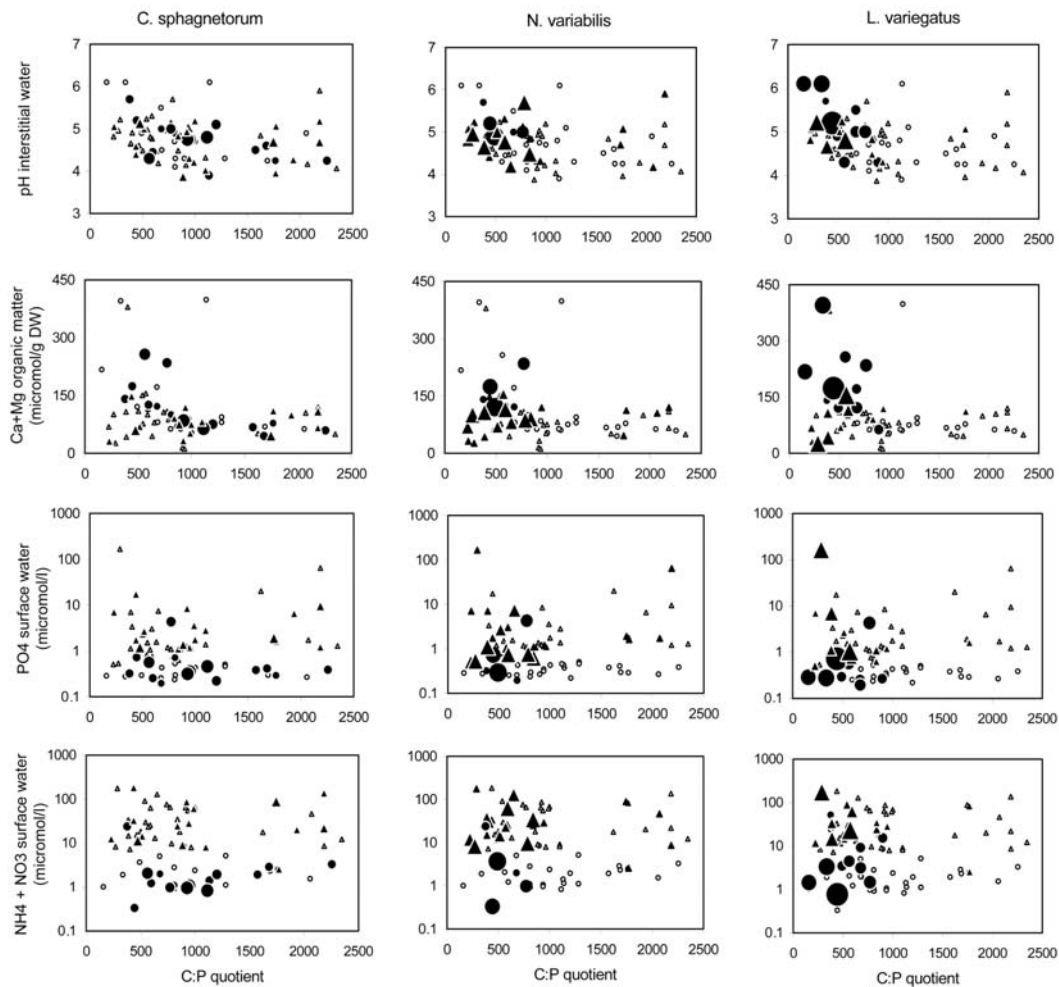


Figure 2. The pH in interstitial water, the Ca+Mg content in dead organic matter, and the concentration of $\text{NH}_4 + \text{NO}_3$ and o-PO_4 in surface water plotted against the C/P quotient of dead organic matter for Estonian ($\circ$) and Dutch (Δ) sampling sites. Filled symbols represent sites in which *C. sphagnetorum*, *N. variabilis* and *L. variegatus* were encountered and the larger the size of the symbol, the higher the abundance of the species. The largest size for >100 individuals per 1m sweep with the pond net and the smallest size for <2 individuals per 1m sweep.

tubificid juveniles were abundant. *Lumbriculus variegatus* was the largest oligochaete species found and accounted for the majority of the oligochaete biomass when present (data not shown).

The ombrotrophic water bodies in the pristine central bog, where only *C. sphagnetorum* was found, had a significantly lower pH and Ca+Mg concentration in the surface and interstitial water, compared to the more minerotrophic parts of the raised bog landscape (Table 2). The Ca+Mg content of dead organic matter was significantly lower, and the C:P quotient was significantly higher in the ombrotrophic bog, compared to the transitional mires, spring, connected pools, and lagg. No statistically significant differences were found for concentrations of $\text{NO}_3 + \text{NH}_4$ and

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Table 3. Chemical data (means $\pm$ standard error) of the sampling sites in Estonia and The Netherlands. Significance of the differences between the means is indicated using the *P*-values of the t-test. n.s. = $P > 0.05$, * = $P < 0.05$, and *** = $P < 0.001$.

	Estonia n=31	The Netherlands n=46	<i>P</i>
surface water			
pH	4.2 $\pm$ 0.1	4.3 $\pm$ 0.1	n.s.
<i>o</i> -PO ₄ (μmol l ⁻¹)	0.5 $\pm$ 0.1	8.0 $\pm$ 3.8	*
NO ₃ + NH ₄ (μmol l ⁻¹)	7.6 $\pm$ 1.7	43 $\pm$ 6.5	***
S (μmol l ⁻¹)	18 $\pm$ 1.0	56 $\pm$ 3.2	***
interstitial water			
pH	4.7 $\pm$ 0.1	4.8 $\pm$ 0.1	n.s.
<i>o</i> -PO ₄ (μmol l ⁻¹)	0.6 $\pm$ 0.3	26 $\pm$ 7.4	***
NO ₃ + NH ₄ (μmol l ⁻¹)	8.5 $\pm$ 2.4	160 $\pm$ 22	***
S (μmol l ⁻¹)	21 $\pm$ 1.5	84 $\pm$ 6.8	***
organic matter			
Ca + Mg (μmol g ⁻¹ DW)	159 $\pm$ 33	132 $\pm$ 16	n.s.
C:N quotient (g g ⁻¹)	29 $\pm$ 1.9	28 $\pm$ 1.6	n.s.
C:P quotient (g g ⁻¹)	997 $\pm$ 91	946 $\pm$ 89	n.s.

Table 4. Results of linear regression of log-transformed species abundance data and chemical data.

	<i>Cognettia sphagnetorum</i>		<i>Nais variabilis</i>		<i>Lumbriculus variegatus</i>	
	R ²	p	R ²	p	R ²	p
surface water						
pH	0.039	n.s.	0.027	n.s.	0.355	p<0.05
<i>o</i> -PO ₄ (μmol l ⁻¹)	0.012	n.s.	0.021	n.s.	0.048	n.s.
NH ₄ +NO ₃ (μmol l ⁻¹)	0.009	n.s.	0.003	n.s.	0.001	n.s.
interstitial water						
pH	0.000	n.s.	0.012	n.s.	0.109	p<0.05
<i>o</i> -PO ₄ (μmol l ⁻¹)	0.019	n.s.	0.001	n.s.	0.000	n.s.
NH ₄ +NO ₃ (μmol l ⁻¹)	0.031	n.s.	0.004	n.s.	0.058	n.s.
organic matter						
Mg+Ca (μmol g ⁻¹ DW)	0.017	n.s.	0.004	n.s.	0.158	p<0.05
C:N quotient (g g ⁻¹)	0.019	n.s.	0.100	p<0.05	0.080	n.s.
C:P quotient (g g ⁻¹)	0.008	n.s.	0.099	p<0.05	0.194	p<0.05

o-PO₄ in surface and interstitial water and for the C:N quotient of organic matter among the parts of the raised bog landscape.

Dutch raised bog remnants

In the Dutch raised bog remnants 7 oligochaete species were found (Table 1). Similar to the Estonian bogs, *C. sphagnetorum*, *N. variabilis*, and *L. variegatus* were the most frequent species. However, in The Netherlands *N. variabilis* was found at more sites than *C. sphagnetorum* and *L. variegatus*, whereas in Estonia *N. variabilis* was found only and exclusively at 5 of the 10 sampling sites inhabited by *L. variegatus*. The sexually reproducing species present in the Estonian lagg zones were not found in the Dutch raised bog remnants. Instead, the naidids *Pristina aequisetata* and *Dero digitata*, reproducing most frequently by paratomy (=budding), and the sexually reproducing species *Stylodrilus heringianus* were found at few sites.

The concentrations of S, NO₃+NH₄ and *o*-PO₄ in the surface and interstitial water of the Dutch sampling sites were significantly higher than in the Estonian sites, whereas there was

no difference in the pH (Table 3). The Ca+Mg content and the C:N and C:P quotients of dead organic matter were not different between Estonian and Dutch sites.

Species' response to water and organic matter quality

The abundance of *L. variegatus* showed a significant relationship with the pH of surface water and interstitial water and with the C:P quotient and Ca+Mg content of dead organic matter (Table 4). The abundance of *N. variabilis* showed a significant relationship with the C:P and C:N quotient of dead organic matter. No significant relationship was found for *C. sphagnetorum*. In both Estonia and The Netherlands *C. sphagnetorum* was found at sites with the C:P and C:N quotient ranging from high to low (Figure 2). In Estonia, *N. variabilis* and *L. variegatus* were only found if the C:P quotient was lower than 1000. This was also true for *L. variegatus* in The Netherlands, except one site where it was present in low abundance (0.75 ind./m). *N. variabilis* was encountered in four Dutch water bodies with a C:P quotient higher than 1000, but it was most abundant if the C:P and C:N quotients were low (lower than 1000 and 30, respectively). Also in the lower range of these quotients *N. variabilis* was in The Netherlands more frequent than in Estonia.

5.4 Discussion

Species occurrence in pristine bogs

The fauna of pristine raised bogs is known to be poor in aquatic oligochaete species (Harnisch 1925 and 1929, Peus 1932). The fragmenting, almost never mature, and acid-tolerant enchytraeid *Cognettia sphagnetorum* is the single oligochaete species able to live in the acidic and extremely nutrient-poor water bodies in the central ombrotrophic raised bog (Table 1). This species is also a typical and abundant inhabitant of peatland soils, such as blanket bogs (Standen & Latter 1977) and Fennoscandian coniferous forests (Huhta et al. 1986). Other oligochaete species are limited to those parts of the bog landscape influenced by minerotrophic alkaline water, like the lagg zones, transitional mires, and bog brooklets.

With over ten species, the lagg zone, influenced by calcareous ground water and characterised by a relatively high abundance of vascular plants and trees, is the most species rich part of the pristine raised bog landscape. Its species assemblage (including *Rhyacodrilus coccineus*, *Tubifex ignotus*, *Tatriella slovenica*, and *Stylodrilus brachystylus*) can be regarded as a relict of the littoral zone fauna of the former lake that has been terrestrialized by the bog formation and has never been affected by eutrophication. *R. coccineus*, *Limnodrilus udekemianus*, *T. ignotus*, and *Spirosperma ferox* occur in many Estonian lakes and rivers, but the lagg community distinctly excludes the ubiquitous tubificids *Limnodrilus hoffmeisteri* and *Tubifex tubifex*, as well as the calciphilous *Psammoryctides barbatus*, also common in most Estonian lakes and rivers. *Tatriella slovenica* is a rare species in Estonia, found mostly in lakes situated between a bog and sandy hills (Timm 1970 and 1999).

Species that reproduce predominantly by sexual modes are absent from the acidic, nutrient poor part of the raised bog (Table 1). Piguet (1906) noted the absence of sexual reproduction in the species *Nais communis* and *Pristina longiseta* in Swiss bog pools. Harnisch (1925) attributed the occurrence of only asexual reproduction to the acidity and extremely low food availability in bogs. The nutrient content and abundance of food is an important factor in the growth rate of oligochaetes (Pasteris et al. 1994). Likewise, detritivorous chironomid and mayfly larvae grow more rapidly and may produce more generations per year if the available detritus contains more

bacteria or has a higher P content (Ward & Cummins 1979, Vos et al. 2002, Frost & Elser 2002). Body growth is constrained by the nutrient content of ingested food, as more food has to be ingested to build up the body biomass when the C:P and C:N quotients are higher (Elser et al. 2000) and mineral content is lower. Streit (1978) found a higher ingestion rate and lower biomass incorporation rate for algae with a low nutritional value. The low nutrient availability may be disadvantageous for sexually reproducing oligochaetes and the acid conditions or absence of specific food components may prevent successful development of eggs and juveniles (Vos et al. 2000). Thus, within the pristine raised bog landscapes in Estonia the pattern in the occurrence and mode of reproduction of aquatic oligochaete species can be linked to the variation in pH and nutrient availability.

Nutrient availability in pristine and polluted raised bogs

In the more minerotrophic parts of the pristine raised bog landscape, pH and buffering capacity (with Ca and Mg as main cations) are significantly higher and the C:P quotient of the dead organic matter is significantly lower than in the ombrotrophic part (Table 2). Therefore, the decomposition rate of the dead organic matter is higher in the minerotrophic parts (Leuven & Wolfs 1988, Kok & Van der Velde 1994, Lamers et al. 1999, Smolders et al. 2002) and hence the nutrient availability is higher. The concentrations of NO_3^- , NH_4^+ , and $o\text{-PO}_4^{3-}$ measured in the surface and interstitial water did not differ significantly between the ombrotrophic and more minerotrophic parts of the bog landscape (Table 2) and no significant relationship was found between the species' abundance and concentrations of N and P in surface and interstitial water (Table 4). This result is not necessarily contradictory to the conclusion that the nutrient availability is higher in the more minerotrophic parts as in pristine (unpolluted) bogs the minerals released by decomposition and mineralisation of the organic matter will be taken up and incorporated in algae, bacteria, mosses, and higher plants (Lamers et al. 2000, Limpens et al. 2003a).

A higher availability of nutrients in the more minerotrophic parts of the raised bog landscape can result in a higher density and a higher nutritional value of algae for algivores (Gulati & DeMott 1997, Elser et al. 2001). Also microbes and vascular plants, like *Menyanthes trifoliata*, *Carex* spp., and trees, can be more abundant. The organic matter formed by these plant species may have a higher nutrient content than the organic matter in the ombrotrophic parts of raised bogs and therefore be more easily decomposed by microbes (Aerts et al. 2001, Scheffer et al. 2001, Smolders et al. 2002, Limpens & Berendse 2003, Tomassen et al. 2003 and 2004a). Next, *Sphagnum* contains a number of structural and non-structural organochemical compounds (such as phenolics) which inhibit microbial activity and further restrict the decomposition of its remnants (Verhoeven & Liefveld 1997, Smolders et al. 2002). Bacteria, algae and many higher plants are characterised by an organic matter quality which is much less resistant to decomposition processes than the *Sphagnum* litter. This implies a better decomposability of the organic matter in transitional mires and lagg zones, resulting in an increased availability of N and P and hence again in an increased growth of bacteria, algae, and leaf-decaying fungi which may stimulate rates of leaf decomposition (Grattan & Suberkropp 2001, Gulis & Suberkropp 2003). A higher abundance and nutrient content of algae, fungi, or bacteria in the minerotrophic parts of the pristine raised bog landscape provide food for more oligochaete species, of which *Lumbriculus variegatus* and *Nais variabilis* are the most abundant.

Due to the prolonged high deposition rates of N and S in The Netherlands, a minerotrophic influence is not necessary anymore for a higher abundance and nutrient content of algae, fungi, or bacteria. Dutch raised bog remnants contain water bodies with both a high availability of

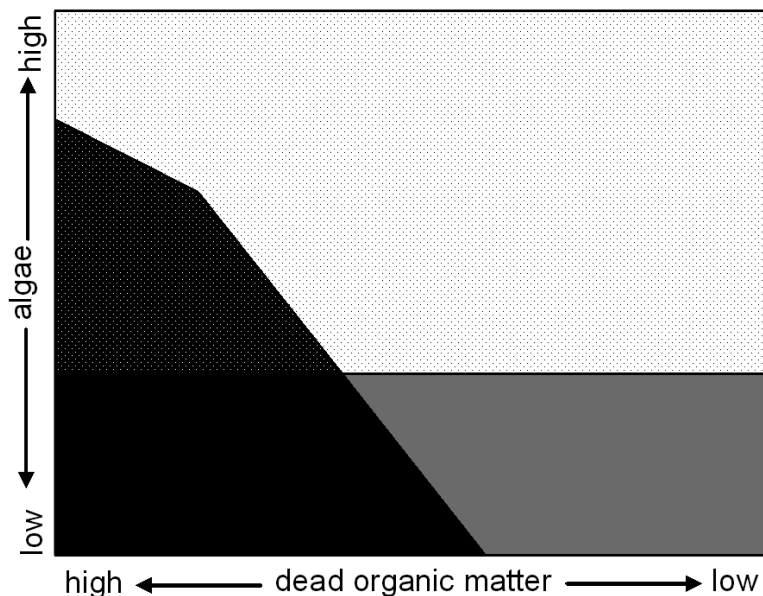


Figure 3. Schematic representation of the combination of dead organic matter quality and decomposition rate vs. quality and abundance of algae in pristine raised bogs (black and grey areas) and raised bogs affected by increased N and S deposition (dotted areas). The white area with black dots represents the situation absent in pristine raised bogs, 'created' by increased N and S deposition.

N and P in the surface water and a high C:P quotient and low Ca+Mg content of dead organic matter (Figure 2). This combination of conditions is absent in pristine Estonian raised bogs and has been 'created' by the prolonged high deposition rates of N and S in The Netherlands. At the same time, the most nutrient-poor conditions of pristine raised bogs are not present anymore in Dutch bog remnants. This situation is schematically represented in Figure 3.

Differential response of oligochaete species to increased nutrient availability

Cognettia sphagnetorum occurred in all parts of Estonian raised bog landscapes (Table 1). No relationship was found between the abundance of this species and nutrient availability in Estonian and Dutch bogs (Table 4; Figure 2). *C. sphagnetorum* is thus not limited by nutrient availability –and is able to live in all situations represented in Figure 3. *N. variabilis* and *L. variegatus* in Estonia were, however, found to be limited to the more minerotrophic parts of the raised bog landscape with a higher nutrient availability. Whereas the frequency of *C. sphagnetorum* and *L. variegatus* was fairly similar in both countries, *N. variabilis* was much more frequent in the Dutch bog remnants than in Estonian bogs (Table 1). These three species differed in their response to water and organic matter variables (Table 4). Probably they use different food items. *C. sphagnetorum* unselectively ingests available organic matter and uses algae and fungi as a food-source (Standen & Latter 1977, Augustsson & Rundgren 1998). Possibly, this species is a primary decomposer (Latter & Howson 1978) and uses also soluble nutrients as food (Springett & Latter 1977). According to Learner et al. (1978) and Harper et al. (1981) *N. variabilis* feeds on bacteria and diatoms, whereas *L. variegatus* feeds on detritus and algae (Moore 1978).

In both Estonia and The Netherlands *L. variegatus* was most abundant in water bodies with a lower C:P quotient and a higher Ca+Mg content of dead organic matter and a higher pH (Table

4; Figure 2) and, thus, with a higher decomposition rate of the organic matter (left part in Figure 3). Increased N and P availability in the surface water in the Dutch bog remnants, favouring growth of algae, seems to be insufficient to increase the frequency of *L. variegatus*. Therefore, we assume that *L. variegatus* predominantly feeds on organic matter and/or on the fungi and bacteria decomposing this matter and that the type of organic matter and its decomposition process are important.

Suitable conditions for *N. variabilis* in Estonian raised bogs are only present if the decomposition rate of organic matter is high (low C:P quotient and higher pH and buffering capacity). Consequently, in Estonia *N. variabilis* is only found in a subset of the water bodies in which *L. variegatus* is present. Contrary to *L. variegatus*, *N. variabilis* in The Netherlands appears to be able to live in water bodies with a higher C:P quotient of organic matter (Figure 2; right-upper area in Figure 3). It may therefore be concluded that *N. variabilis* is not dependent on organic matter with a high decomposition rate and the allied microbes, but responds to the abundance and nutritional value of diatoms or other algae, which are affected by N and P availability in surface water. However, it can not be precluded that in the Dutch bog remnants the prolonged high deposition rates of N and S and the increase in higher plants and algae have led to a new type of dead organic matter that is more easily decomposed by microbes, although it still may have a relatively high C:P quotient. The organic compounds produced during decomposition of this new type of organic matter may not always possess a sufficient nutritional value for *L. variegatus*. More frequently *N. variabilis* may profit from the increased abundance of bacteria and/or (micro)algae resulting from the higher availability of nutrients (N and P) in Dutch bog remnants. Therefore, in The Netherlands *N. variabilis* can be found in water bodies that are not inhabited by *L. variegatus*.

5.5 Conclusion

The most nutrient poor water bodies in the pristine central raised bog are only inhabited by *Cognettia sphagnetorum*. Other oligochaete species in pristine Estonian raised bogs, of which *Nais variabilis* and *Lumbriculus variegatus* are most abundant, are limited to more minerotrophic water bodies with an increased decomposition rate of organic matter and a higher nutrient availability. However, in Dutch raised bog remnants the occurrence of oligochaetes is not limited anymore by N and P availability, due to increased atmospheric N and S deposition. *C. sphagnetorum*, *N. variabilis*, and *L. variegatus* respond differently to the increased availability of N and P in The Netherlands, probably because of differences in their diets. In Estonia species that reproduce sexually are limited to the minerotrophic parts of the bog landscape (springs and laggs). Most of the lagg zone species were not found in Dutch bog remnants as this part of raised bog landscapes has long been cultivated and the specific conditions of laggs are not present anymore in almost all Dutch bog remnants. Overall, it can be concluded that the degradation of Dutch raised bogs has resulted in the loss of both the nutrient-poor parts of the landscape and the special lagg conditions.

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Chapter 6

Methane as a carbon source for the food web in raised bog pools

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Submitted

◀ Food web relations may be complex. All herbivores, like this butterfly, grow by eating plants, but some of them are eaten by insectivorous plants.

Abstract

Raised bog pools are extremely nutrient poor and rich in humic substances, limiting primary production. To assess the base of the invertebrate food web in bog pools stable isotopic signatures of primary producers, dead organic matter, and invertebrates, as well as the composition and stable carbon isotope ratio of phospholipid fatty acids (PLFAs) were measured. The stable isotopic signatures showed the presence of multiple trophic levels and a differential use of basal food sources by the invertebrates, both between different species and within species, among different individuals and size classes. Carnivorous and omnivorous invertebrates assimilated polyunsaturated fatty acids (PUFAs) derived from algae, and possibly macrophytes, as well as fatty acids that are specific for methane oxidizing bacteria (MOB). A considerable part of the bacterial biomass conveyed to higher trophic levels in the bog pools likely originates from MOB. Protozoa and zooplankton synthesizing PUFAs commonly used as biomarkers for algae may play a role in this pathway. Pelagic zooplankton seems to rely more on bacteria, whereas for insects algae are more important. Periphyton was the basal food source most depleted in ^{13}C and inferred to sustain at least half the food web. The relatively depleted $\delta^{13}\text{C}$ values of PUFAs in invertebrates point to the use of algae that possibly derived carbon from MOB. Therefore, depleted $\delta^{13}\text{C}$ values of invertebrates do not necessarily implicate a direct pathway between MOB and these invertebrates, but algal food sources forming an intermediate level.

6.1 Introduction

Heterotrophic organisms are sustained by living or dead biomass. This organic matter can be locally produced or imported from elsewhere. In pristine raised bogs, primary production is strongly nutrient limited and the nutrient content of the dominant *Sphagnum* mosses and vascular plants is extremely low (Aerts et al. 1999). Pools are a significant feature of raised bogs (Belyea & Lancaster 2002), harbouring a large biodiversity of aquatic macroinvertebrates (Desrochers & Van Duinen 2006, Verberk et al. 2006). In these pools primary production by submerged macrophytes and algae is further constrained by low levels of light, resulting from a high concentration of humic substances (Karlsson et al. 2009). As a consequence of the low nutrient content of living and dead organic matter in bog pools, consisting mostly of mosses and vascular plants, the decomposition rate of dead organic matter is very low, something which is compounded by the acidic conditions in raised bogs (Belyea 1996, Smolders et al. 2002). The limited primary production and low nutritional value of living and dead plants give rise to the question what basal food sources sustain the food web in raised bog pools.

Run-off water providing organic carbon sources could potentially provide another basal food source to sustain the food web in raised bog pools. In lakes, the relative importance of these allochthonous organic carbon sources to the food web increases with decreasing lake trophy and decreasing phytoplankton production (Grey et al. 2000, Pace et al. 2007). Contrary to lakes and streams, raised bog pools are isolated from other water bodies and do not have a large catchment area that could supplement the food web with allochthonous organic carbon and other nutrients. Bog pools are embedded in peat, constantly releasing humic substances. Concerning bog pools, Rydin & Jeglum (2006) suggested bacteria feeding on dissolved humic substances as a second basal food source, in addition to photosynthesis. Jones (1992) described humic substances as an important carbon source in planktonic food chains in lakes in which primary production of algae is limited by oligotrophy or humic substances. Although humic substances are highly recalcitrant to microbial degradation, Tranvik (1988) found that lakes with a high content of

humic substances could support a higher bacterial biomass than clearwater lakes due to their larger pools of dissolved organic carbon (DOC).

Biogenic methane could be a third basal carbon source. In bogs methane is produced during the decomposition of peat (Raghoebarsing et al. 2005). Methane-derived carbon can contribute to the food web via methanotrophic bacteria, which are found to be ingested by zooplankton (Bastviken et al. 2003, Taipale et al. 2007), chironomid larvae (Jones et al. 2008), and caddisfly larvae grazing their own cases (Trimmer et al. 2009).

A powerful tool to distinguish between the potential food sources and to determine the configuration of food webs is dual stable isotope analysis ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of producers and consumers. This approach is based on a predictable change in the natural abundance stable isotopes composition between the different trophic levels (DeNiro & Epstein 1978, Minagawa & Wada 1984) and has been applied in a wide range of ecosystems. However, the extent to which the pathways conveying organic matter to consumers and their predators can be inferred solely from stable isotopic signatures depends on the variation and distinctness of the isotopic signatures of basal food sources. In addition, the isotopic signature of a consumer can result from the consumption of a single food source, but more realistically from the consumption of a mixture of two or more food sources. One way to gain a better understanding of the relative importance of basal food sources in food webs is to combine the analysis of natural abundance stable isotopes composition with analyses of phospholipid-derived fatty acids (PLFAs) composition (Kharlamenko et al. 2001, Perga et al. 2006, Van den Meersche et al. 2009). The approach using PLFAs is based on the specific PLFA composition of bacteria and algae and on the inability of animals to synthesize specific PLFAs and essential polyunsaturated fatty acids (Kharlamenko et al. 2001).

To our knowledge, Kato et al. (2010) and Van Duinen et al. (2006a) performed the only food web studies applying stable isotopes analyses in a temperate bog. Kato et al. (2010) focussed on a hummock-hollow complex rather than raised bog pools. Interestingly, both studies highlighted a missing basal carbon source. Kato et al. (2010) found dead leaf stalks of a dominant vascular plant (*Menyanthes trifoliata*) and benthic particulate organic matter to be the most likely potential food sources for aquatic and terrestrial detritivores, but aquatic predators seemed to rely also on another unknown basal food source, enriched in ^{13}C compared to the benthic particulate organic matter. In our previous study in raised bog pools (Van Duinen et al. 2006a) we inferred that the missing basal carbon source should be more depleted in ^{13}C compared to the living macrophytes, filamentous algae and dead organic matter present in these pools, but we were unable to verify its identity. This depleted food source could be based on methane, which is the only component carrying a very negative $\delta^{13}\text{C}$ value (Boschker & Middelburg 2002). The role of methane in freshwater food webs has recently attracted much attention (Jones & Grey 2011).

Here, we revisit the enigma of a missing basal carbon source and investigate the food web of three pools in the raised bog Nigula, Southwest Estonia, by means of analysis of both stable isotopes and PLFAs to assess if this food web is sustained by the primary producers that dominate the plant biomass in these pools (the macrophytes *Sphagnum* mosses and vascular plants), their dead organic matter, and dissolved organic substances, or that algae, or methanotrophic bacteria contribute to the food web, as well. Specifically, we address the following questions:

1. Do the isotopic signatures of the aquatic invertebrates of different trophic levels indicate

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use of macrophytes, their dead organic matter, dissolved organic substances, algae, or other basal food sources?

2. Can the PLFA composition of aquatic invertebrates be used to infer the trophic pathways in the food web in raised bog pools?

6.2 Methods

Study area

The three bog pools (N1, N2 and N3) were situated in the pristine raised bog massif of Nigula Nature Reserve, Southwest Estonia (Figure 1). At each of the three pools samples of surface water, sediment pore water and sedimented organic matter (SOM) were collected in May 2001 and September 2002 to assess pH and the concentrations of nutrients and other components. For further details about the methods used for analyses see Van Duinen et al. (2003 and 2006b). The concentration of dissolved organic carbon (DOC) in surface water was analysed in samples collected in December 2006 and July 2007. For each pool nutrient content and other background data are presented as averages of the two sampling periods (Table 1).

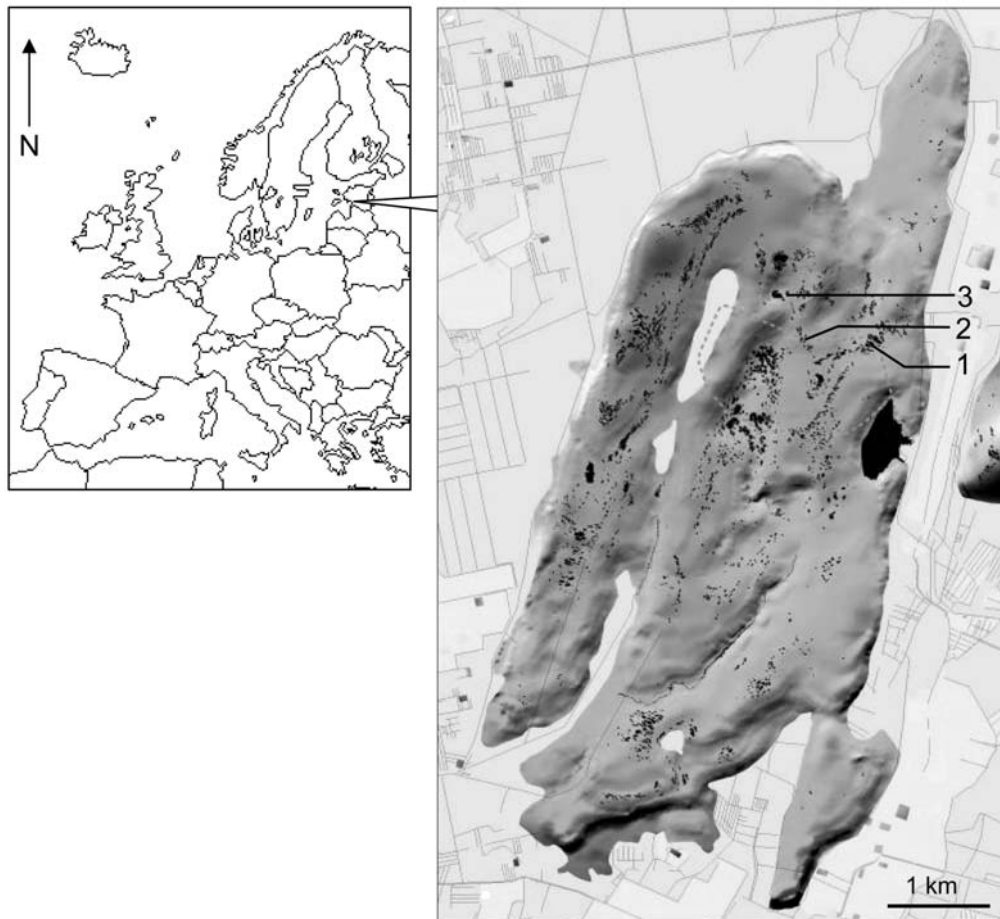


Figure 1. Geographical location of the three raised bog pools studied in Nigula Nature Reserve, Southwest Estonia.

Table 1. Average ($\pm$ standard deviation) quality data of surface water, sediment pore water and sedimented organic matter at the sampling sites. N=2 sampling periods.

	Site	Nigula 1	Nigula 2	Nigula 3
Surface water				
pH		3.9 $\pm$ 0.1	3.9 $\pm$ 0.2	4.0 $\pm$ 0.1
o-PO ₄ (μ mol l ⁻¹)		0.23 $\pm$ 0.10	0.17 $\pm$ 0.16	0.28 $\pm$ 0.01
NO ₃ +NH ₄ (μ mol l ⁻¹)		4.8 $\pm$ 2.5	9.3 $\pm$ 4.0	10.7 $\pm$ 9.0
Ca (μ mol l ⁻¹)		17.2 $\pm$ 8.9	23.3 $\pm$ 12.0	25.3 $\pm$ 9.9
Cl (μ mol l ⁻¹)		58.7 $\pm$ 5.3	90.5 $\pm$ 31.3	71.3 $\pm$ 5.9
Dissolved inorganic carbon (DIC) (μ mol l ⁻¹)		22.6 $\pm$ 31.9	44.7 $\pm$ 25.5	32.6 $\pm$ 40.2
Dissolved organic carbon (DOC) (μ mol l ⁻¹)		1654 $\pm$ 98	1925 $\pm$ 59	1671 $\pm$ 457
Sediment pore water				
pH		4.7 $\pm$ 0.7	4.6 $\pm$ 0.6	4.5 $\pm$ 0.2
o-PO ₄ (μ mol l ⁻¹)		0.18 $\pm$ 0.06	0.16 $\pm$ 0.11	0.47 $\pm$ 0.65
NO ₃ +NH ₄ (μ mol l ⁻¹)		2.2 $\pm$ 2.6	12.7 $\pm$ 14.5	26.6 $\pm$ 17.6
Ca (μ mol l ⁻¹)		58.2 $\pm$ 44.5	55.1 $\pm$ 37.7	51.0 $\pm$ 26.7
Cl (μ mol l ⁻¹)		55.2 $\pm$ 8.9	58.5 $\pm$ 6.9	63.0 $\pm$ 18.3
Dissolved inorganic carbon (DIC) (μ mol l ⁻¹)		42.3 $\pm$ 14.9	38.9 $\pm$ 14.9	54.2 $\pm$ 29.1
Sedimented organic matter				
C:P (g g ⁻¹)		1293 $\pm$ 636	1309 $\pm$ 403	652 $\pm$ 347
C:N (g g ⁻¹)		29.8 $\pm$ 17.7	24.7 $\pm$ 15.1	17.2 $\pm$ 5.7
Ca (μ mol g ⁻¹ DW)		72.9 $\pm$ 25.1	51.3 $\pm$ 10.0	66.4 $\pm$ 14.3

Sampling and analyses of stable isotope ratios

Plants (filamentous algae, mosses and vascular plants) and aquatic macroinvertebrates were collected in September 2002 at the three pools. SOM was collected from the peat bottom by means of a plankton net with a mesh size of 45 μ m. Zooplankton was collected from the open water by means of a plankton net with a mesh size of 115 μ m and light traps. As an additional potential source to aquatic invertebrates, invertebrates flying and walking around the bog pools and eventually drowning in the pools, were collected in August 2006. Periphyton (mainly consisting of algae) was collected by scraping from plastic sheets after rinsing with demineralised water. These sheets (30x25cm) hung vertically in the water bodies for one month in August-September 2007 with their upper end close to the water surface. In these pools fishes do not occur and amphibians are rare. Gut contents were not removed from invertebrates, as trials with several species showed that they did not empty their guts within two or more days of living in filtered surface water of bog pools. Collected invertebrates were sorted, washed with demineralised water and kept in a fridge until identification to species or genus level. Identified material was dried for 24 hours at 70°C and subsequently ground, using liquid nitrogen. Large macroinvertebrates were analysed individually, whereas smaller individuals were pooled per species. Carbon and nitrogen isotopic composition of each sample was determined in duplo or triplo with a Carlo Erba NA 1500 elemental analyzer coupled online via a Finnigan Conflo III interface with a ThermoFinnigan DeltaPlus mass-spectrometer.

Surface water samples for analysis of the $\delta^{13}\text{C}$ value of DOC were collected in December 2006 and July 2007 by filtering surface water over a filter with mesh size 0.2 μ m (Schleicher & Schuell FP 030/3) and adding 100 μ L 50% H₃PO₄ to 40 mL water sample. The carbon isotopic

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composition of dissolved organic carbon has been measured using a high-performance-liquid-chromatograph coupled via a LC-Isolink interface to an isotope-ratio mass spectrometer (Delta V Advantage IRMS, Thermo). The technique of the Isolink interface is based on the wet oxidation of organic analytes with peroxodisulfate under acidic conditions. The CO₂ produced is subsequently separated from the mobile phase in a capillary gas exchanger flushed with helium gas, dried before introduction into the IRMS (Boschker et al. 2008).

Stable isotope data are presented as the relative difference between the ratios of the sample and the standards, using the following formula:

$$\delta R = [(R_{\text{sample}} / R_{\text{standard}}) - 1] \times 1000$$

where $R = {}^{13}\text{C}/{}^{12}\text{C}$ or ${}^{15}\text{N}/{}^{14}\text{N}$. $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ is the per mille (‰) deviation of the sample from their isotope standards, that are Vienna PeeDee belemnite for $\delta^{13}\text{C}$ and atmospheric N₂ for $\delta^{15}\text{N}$. Average reproducibility based on replicate measurements of samples and internal standards Sucrose (IAEA-CH-6) for $\delta^{13}\text{C}$ and Ammonium sulphate (IAEA-N-2) for $\delta^{15}\text{N}$ was <0.2‰.

Estimation of the contribution of basal carbon sources

We estimated the feasible contributions of the different potential basal carbon sources (SOM, DOC, submerged *Sphagnum*, vascular plants, filamentous green algae, and periphyton) for each trophic group of invertebrates by means of isotope mixing models for $\delta^{13}\text{C}$ to get an indication of the contribution of these carbon sources in sustaining the higher trophic levels. Invertebrates were classified in trophic groups according to Nilsson (1996, 1997) and references therein, Vallenduuk & Moller Pillot (2007), Moller Pillot (2009), and Higler (2005). For each group of basal carbon sources and each trophic group of invertebrates (carnivores, omnivores, and herbi-detritivores; the latter including species classified as herbivores, detritivores and herbi-detritivores) the average $\delta^{13}\text{C}$ value was calculated and used as input to the mixing model. We used IsoSource version 1.3.1 (Phillips & Gregg 2003), creating all possible combinations of proportions of the six potential basal carbon sources, with increments of these proportions set at 1%. Combinations that sum to the average $\delta^{13}\text{C}$ value of the trophic group within a tolerance of 0.1‰ were considered to be feasible solutions. We assumed trophic fractionation to be negligible.

Lipid analyses and stable isotope analysis of PLFAs

SOM, pelagic zooplankton, and several mostly carnivorous insect species were collected in the pools in August 2006 and subsequently freeze dried and ground. Benthic macrofauna was removed from the SOM samples. Lipid analyses and stable isotope analyses of PLFAs were performed as described by Mohanty et al. (2006). Lipids were extracted from 0.5 g of the sedimented organic matter and 0.1 g of the invertebrate material with a Bligh-Dyer extraction procedure as modified and described by Boschker et al. (1998, 2001). The lipid extract was fractionated on silicic acid into different polarity classes by sequential elution with chloroform, acetone, and methanol. The methanol fraction containing the PLFA was derivatized using mild-alkaline methanolysis to yield fatty acid methylesters (FAME). FAME standards of both C12:0 and C19:0 were used for calculating retention indices and for FAME quantification. Identification of FAME was based on retention time data with known standards. Additional identification was gained by GC-mass spectrometry (GC-MS) using a Thermo Finnigan TRACE GC-MS system. For identification of methanotroph-specific PLFA, extracts of cultures of *Methylomonas methanica* SI NCIMB 11130, *Methylobacterium album* NCIMB 11123, *Methylobacter luteus* NCIMB 11914, *Methylocystis parvus* NCIMB 11129, *Methylosinus trichosporium* NCIMB 11131, and *Methylosinus sporium*

NCIMB 11126 were used as references. PLFA nomenclature used is as described by Guckert et al. (1985). PLFAs are designated by the number of carbon atoms. The degree of unsaturation is indicated by a number separated from the chain length by a colon. This number is followed by ω xc or ω xt, where x indicates the position of the double bond nearest to the aliphatic end (ω) of the molecule and c and t indicate a cis and trans stereoisomeric position of the double bond on the molecule. The prefixes i and a refer to iso and anteiso branching. The prefix 10Me refers to methyl branching at the 10th carbon from the carboxyl group. The prefix br indicates an unknown branching. The prefix cy refers to cyclopropyl rings. PLFAs with unknown molecule structure are referred to using the equivalent chain length (ECL) expressing their retention time relative to those of known straight-chain saturated FAME.

FAME concentrations were determined using a GC-FID system (Thermo Finnigan TRACE GC) equipped with a polar capillary column (SGE, BPX-70; 50 m by 0.32 mm by 0.25 μ m), using the following oven conditions: initial temperature of 50 °C for 1 min, and then the temperature was programmed to 130 °C using a ramp of 40 °C min⁻¹ followed by an increase to 230 °C with a ramp of 3 °C min⁻¹.

Stable carbon isotope ratios for individual FAME were determined using a Varian 3400 GC equipped with an ATAS Optic 2 programmable direct thermal desorption injection system. The GC was coupled via a type II combustion interface to a Finnigan Delta S isotope ratio mass spectrometer. The same polar capillary column was used as for FAME identification and quantification on the GC-FID and GC-MS systems. The oven temperature for the GC-isotope ratio mass spectrometry analyses was as follows: initial temperature of 50 °C for 4 min, and then the temperature was programmed to 130 °C using a ramp of 30 °C min⁻¹, which was immediately followed by an increase to 200 °C using a ramp of 6 °C min⁻¹, a subsequent increase to 220 °C using a ramp of 5 °C min⁻¹, and a final increase to 250 °C using a ramp of 20 °C min⁻¹. The sample was injected into the direct thermal desorption system at 50 °C, after which the temperature was programmed to 260 °C with a ramp of 10 °C s⁻¹. PLFAs with a relative concentration < 0.1% are disregarded. $\delta^{13}\text{C}$ values of PLFAs with a relative concentration < 1% are regarded as unreliable and not presented here. The potential affiliated biota of the PLFAs found in SOM and invertebrates was taken from Boschker & Middelburg (2002), Dijkman & Kromkamp (2006) and Taipale et al. (2009), accomplished with various other papers mentioned in the results and discussion sections.

6.3 Results

Stable isotopic signatures

The various invertebrate species collected in the three bog pools differed in their $\delta^{13}\text{C}$ values (Table 2), indicating a differential use of basal carbon sources, and in their $\delta^{15}\text{N}$ values, indicating the presence of multiple trophic levels (Figure 2). The living and dead plant material showed the lowest $\delta^{15}\text{N}$ values. Most aquatic invertebrate species collected were carnivorous according to literature (Table 2). The highest $\delta^{15}\text{N}$ values, in the range of 1.2 to 10.3‰, were found for the heteropterans *Notonecta glauca*, *Nepa cinerea* and *Ranatra linearis*, the water spider *Argyroneta aquatica*, and the coleopterans *Dytiscus dimidiatus*, *Dytiscus lapponicus*, *Acilius canaliculatus* and *Acilius sulcatus*, which are known to be top-predators. The $\delta^{15}\text{N}$ values of corixid species and dipteran, dragonfly, damselfly, mayfly, and caddis fly nymphs and larvae ranged between -2.0 and 2.8‰. The invertebrates with the lowest $\delta^{15}\text{N}$ values were found among the invertebrate species known as herbivores, herbi-detritivores or omnivores, e.g. zooplankton in pool N1

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Table 2. Values (‰) of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ of sedimented and dissolved organic matter, plants and invertebrates of the three bog pools N1, N2 and N3. Invertebrates are arranged according to trophic group indicated in column T (c = carnivore, d = detritivore, h = herbivore, hd = herbi-detritivore, o = omnivore) and subsequently to taxonomical group indicated in column 'Tax.' (Odo=Odonata, Het=Heteroptera, Col=Coleoptera, Meg=Megaloptera, Dip=Diptera, Tri=Trichoptera, Ara=Aranea, Cru=Crustacea, Eph=Ephemeroptera). In column # the number 1 indicates that specimens of the species or higher taxon were analysed individually, otherwise individuals were pooled per species or higher taxon.

Species	#	Tax.	T	N1		N2		N3	
				$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$
Sedimented organic matter (SOM)				-2.52	-25.97	-3.21	-26.36	-2.66	-25.41
Dissolved organic carbon (DOC)					-27.58		-27.57		-26.63
Periphyton				-3.24	-27.42	-1.80	-31.53	-3.56	-34.69
Branched green algae				-3.40	-25.71			-0.75	-23.33
Filamentous green algae				-2.29	-24.35	-2.37	-19.42	-2.15	-22.40
Leaf moss (submerged)				-3.38	-28.24	-3.05	-27.89	-0.84	-25.41
<i>Sphagnum cuspidatum</i> (submerged)				-2.39	-26.73	-2.11	-26.58	1.22	-23.92
<i>S. magellanicum</i> (from lawn)				-3.35	-24.50	-4.03	-24.76	-5.14	-27.44
<i>S. magellanicum</i> (submerged)				-2.48	-24.53	-3.60	-24.59		
<i>Carex limosa</i> (living plant)						-5.72	-27.79	-0.87	-27.27
<i>Carex rostrata</i> (dead leaves)								-0.85	-26.58
<i>Carex rostrata</i> (water)								-0.35	-26.75
<i>Carex rostrata</i> (roots)								-3.17	-26.69
<i>Scheuchzeria palustris</i>						-6.06	-25.57	-2.65	-26.19
<i>Rhynchospora alba</i>				-3.55	-27.40				
<i>Utricularia minor</i>						-1.18	-22.51	-1.41	-21.06
<i>Betula</i> spec. fallen leaves				-7.43	-29.62				
Zygoptera nymphs		Odo	c	2.05	-30.41	1.13	-27.46	1.61	-27.39
Anisoptera young nymphs		Odo	c	0.44	-25.37	0.41	-25.89	0.48	-25.67
Anisoptera last stage nymphs		Odo	c	0.88	-27.95				
<i>Anax imperator</i> nymphs last stage		Odo	c			2.77	-29.62		
<i>Anax imperator</i> young nymphs		Odo	c			2.24	-28.09		
<i>Aeshna</i> spec. nymphs		Odo	c	1.01	-26.35			0.39	-27.87
<i>Libellula</i> spec. nympe 1	1	Odo	c	0.87	-31.18			-0.44	-26.92
<i>Libellula</i> spec. nympe 2	1	Odo	c	-0.31	-30.26			0.18	-27.77
<i>Leucorrhinia</i> spec. nymphs		Odo	c	0.18	-25.51				
Libellulidae nymphs		Odo	c	0.65	-30.12	0.48	-29.32	-0.77	-27.77
<i>Cymatia bonndorffii</i>		Het	c	0.77	-30.97	1.47	-32.77	-0.38	-30.02
<i>Notonecta glauca</i> 1	1	Het	c	1.22	-28.52				
<i>Notonecta glauca</i> 2	1	Het	c	2.24	-28.62	2.67	-25.32		
<i>Notonecta glauca</i> 3	1	Het	c	10.33	-32.07	2.50	-28.91		
<i>Notonecta glauca</i> 4	1	Het	c	8.19	-34.70	2.29	-29.19	1.01	-28.05
<i>Ilyocoris cimicoides</i> 1	1	Het	c	0.73	-25.26	0.66	-28.21	-0.05	-24.58
<i>Ilyocoris cimicoides</i> 2	1	Het	c	0.46	-24.80				
<i>Nepa cinerea</i>	1	Het	c	4.68	-27.61				
<i>Ranatra linearis</i>	1	Het	c	4.15	-30.30	6.65	-26.18		
<i>Gerris</i> spec.		Het	c			1.76	-25.49		
<i>Acilius canaliculatus</i> 1	1	Col	c	2.26	-33.89	4.08	-29.93	2.41	-28.68
<i>Acilius canaliculatus</i> 2	1	Col	c	2.19	-28.85	3.67	-28.93		
<i>Acilius canaliculatus</i> 3	1	Col	c	3.41	-31.14				
<i>Acilius canaliculatus</i> 4	1	Col	c	2.24	-28.71				

Methane in bog pool food web

Species	#	Tax.	T	N1		N2		N3	
				$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$
<i>Acilius canaliculatus</i> 5	1	Col	c	6.31	-28.82				
<i>Acilius canaliculatus</i> 6	1	Col	c	3.13	-27.25				
<i>Acilius sulcatus</i> 1	1	Col	c	1.77	-30.33	2.21	-29.19		
<i>Acilius sulcatus</i> 2	1	Col	c	1.74	-29.77				
<i>Acilius sulcatus</i> 3	1	Col	c	2.04	-32.55				
<i>Acilius sulcatus</i> 4	1	Col	c	3.80	-30.75				
<i>Acilius sulcatus</i> 5	1	Col	c	4.28	-30.03				
<i>Acilius sulcatus</i> 6	1	Col	c	1.89	-31.22				
<i>Dytiscus dimidiatus</i> 1	1	Col	c	4.24	-29.00	5.33	-29.11		
<i>Dytiscus dimidiatus</i> 2	1	Col	c	5.50	-28.37	3.64	-30.60	2.63	-38.57
<i>Dytiscus lapponicus</i> 3	1	Col	c	1.97	-29.83	1.87	-29.24		
<i>Dytiscus lapponicus</i> 4	1	Col	c	0.94	-29.84	1.84	-29.12		
<i>Dytiscus lapponicus</i> 5	1	Col	c			1.84	-28.97		
<i>Graphoderus cinereus</i>		Col	c					0.62	-28.10
<i>Hyphydrus ovatus</i>		Col	c					0.23	-27.03
<i>Ilybius subaeneus</i>		Col	c	0.95	-31.24	0.35	-26.54		
<i>Gyrinus</i> spec.		Col	c	3.89	-32.47				
Dytiscidae larvae		Col	c					0.40	-24.33
<i>Sialis</i> spec. larvae		Meg	c					-1.13	-29.94
<i>Chaoborus</i> spec. larvae		Dip	c			2.51	-29.90		
<i>Ablabesmyia</i> spec. larvae		Dip	c					-0.33	-28.24
Polycentropodidae larvae		Tri	c	1.30	-27.93	0.59	-27.68	1.30	-28.19
<i>Argyroneta aquatica</i> (small)		Ara	c	2.67	-27.57	2.97	-25.81	2.10	-24.76
<i>Argyroneta aquatica</i> (large)		Ara	c			3.07	-28.91		
Hydracarina		Aca	c					3.78	-27.88
<i>Asellus aquaticus</i>		Cru	d					-1.22	-26.53
<i>Leptophlebia vespertina</i> nymphs		Eph	hd			-1.12	-28.70	-1.12	-25.94
<i>Psectrocladius</i> spec. larvae		Dip	hd	-1.56	-24.25				
Chironominae larvae		Dip	hd	0.56	-26.28				
<i>Chironomus</i> spec. larvae		Dip	hd					-2.04	-28.43
<i>Phalacrocer a replicata</i> larvae		Dip	h			-0.37	-27.27	-0.12	-25.32
<i>Sigara scotti</i>		Het	o	0.48	-29.17			-1.40	-28.61
<i>Sigara semistriata</i>		Het	o					-0.45	-26.17
<i>Hesperocorixa linnaei</i>		Het	o					2.04	-29.19
<i>Hesperocorixa sahlbergi</i>		Het	o	2.66	-30.24				
<i>Glaenocoris propinqua</i>		Het	o	1.35	-31.20				
<i>Corixa dentipes</i>		Het	o	1.50	-30.33			-1.07	-30.63
<i>Phryganea bipunctata</i> larvae		Tri	o	0.07	-26.62			-0.39	-26.27
Zooplankton				-0.94	-33.27			2.81	-31.36

(dominated by the microcrustaceans *Bosmina* spec., *Chydorus sphaericus* and copepodites), *Leptophlebia vespertina* nymphs in pools N2 and N3, and larvae of the chironomid genera *Psectrocladius* and *Chironomus* in pools N1 and N3.

Individuals of the large predatory invertebrate species *Acilius canaliculatus*, *Acilius sulcatus*, *Dytiscus dimidiatus*, *Dytiscus lapponicus* and *Notonecta glauca* were analysed separately. Various individuals of the same species captured in the same water body differed strongly in isotopic signature for both C and N (Table 2).

Many of the invertebrates were more depleted in ^{13}C (< -28‰) than the dominant primary producers (vascular plants, mosses, filamentous and branched green algae), their dead organic

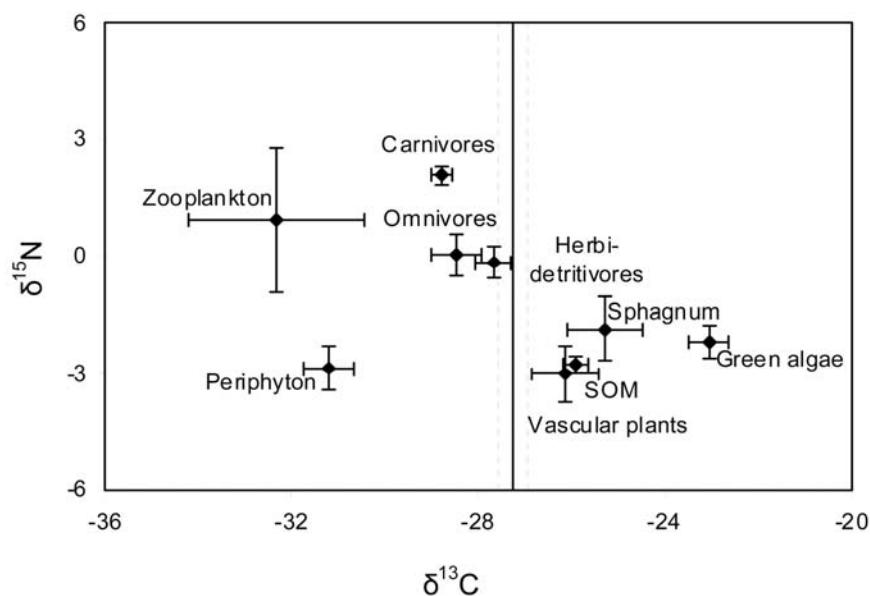


Figure 2. Average values $\pm$ SE (‰) of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ of different groups of primary producers, sedimented organic matter (SOM), and different trophic groups of invertebrate species in the bog pools N1, N2, and N3. The vertical lines indicates the average $\delta^{13}\text{C}$ ($\pm$ SE) of dissolved organic matter (DOC) of the three bog pools. The classification of invertebrates in the different trophic groups is given in Table 2.

matter, and DOC (Figure 2). Periphyton (mainly consisting of green algae) was the most depleted potential basal carbon source found. The periphyton varied considerably in their $\delta^{13}\text{C}$ values between the three bog pools (Table 2). The lowest $\delta^{13}\text{C}$ value of periphyton, found in N3 (-34.69‰), could account for the $\delta^{13}\text{C}$ value of at least the more depleted half of the invertebrate food web, assuming an enrichment (less negative) of 0 to 1‰ for the $\delta^{13}\text{C}$ values between trophic levels (e.g. Post 2002, McCutchan et al. 2003). The mixing models indicated that periphyton contributes on average 55% to the trophic group of the carnivores and at least 44% (1% percentile), and a bit less in the case of the omnivores and herbi-detritivores. The contribution estimated for the other potential basal carbon sources was considerably lower (Table 4). For zooplankton the feasible contribution was not assessable as their $\delta^{13}\text{C}$ was more depleted than those of the potential basal carbon sources.

The $\delta^{13}\text{C}$ values of invertebrates flying and walking around the bog pools exceeded -28‰ , with the exception of several imagines of Trichoptera, Nematocera, and the damselfly *Enallagma cyathigerum* (Table 3), whose aquatic larvae or nymphs have developed in the bog pools. The $\delta^{15}\text{N}$ values of many of these invertebrates collected around the pools overlapped with those of the aquatic invertebrates. Assuming a trophic enrichment (less negative) of about 3‰ for the $\delta^{15}\text{N}$ values (according to Minagawa & Wada (1984) and Post (2002) and confirmed by the average $\delta^{15}\text{N}$ values of the trophic levels in this study (Figure 2)) and of 0 to 1‰ for the $\delta^{13}\text{C}$ values, it is unlikely that these invertebrates are a major component in sustaining the aquatic food web.

PLFA composition and stable carbon isotope ratio

The PLFAs characteristic for methane oxidizing bacteria type I (MOB I: 16:1 ω 8c and 16:1 ω 5t;

Table 3. Values (‰) of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ of invertebrates (imagines, unless otherwise indicated) walking and flying around the bog pools. The taxa are arranged from low to high $\delta^{13}\text{C}$.

Species	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$
<i>Enallagma cyathigerum</i>	2.90	-33.64
Trichoptera	2.66	-31.48
Trichoptera	3.65	-30.48
Nematocera	2.08	-30.43
Trichoptera (Leptoceridae)	2.28	-29.72
Lepidoptera larva	-3.50	-28.55
Lepidoptera (moth)	-4.32	-28.20
Lycosidae	2.10	-27.84
Trichoptera (<i>Limnephilus</i> spec.)	1.22	-27.77
<i>Tetragnatha</i> spec.	2.37	-27.76
Phylalidae	2.96	-27.74
Lycosidae	1.91	-27.53
Lepidoptera (moth)	0.71	-27.23
<i>Sympetrum danae</i>	0.50	-27.00
Brachycera	4.33	-26.88
Diplopoda	-2.88	-26.86
Nabidae	-1.11	-26.77
<i>Hippodamia</i> spec.	0.04	-26.75
Aranaeidae	2.98	-26.56
<i>Bombus jonellus</i>	-0.59	-26.41
Trichoptera (<i>Limnephilus</i> spec.)	-1.04	-26.34
<i>Pterostichus minor</i>	-0.40	-26.24
<i>Metrioptera brachyptera</i>	-4.34	-26.19
Thomisidae	4.08	-26.00
<i>Formica</i> spec.	0.51	-25.73
Lepidoptera larva	-4.29	-25.43
<i>Proclissiana eunomia</i>	-8.67	-25.40
Calliphoridae	7.10	-24.98
Muscidae - Coenosiinae	4.60	-24.69
Limoniinae	2.41	-24.34

Table 4. Means and 1 and 99 percentiles of the feasible contribution of potential basal carbon sources to the different trophic groups of invertebrates. The classification of invertebrate species in the trophic groups is given in Table 2.

	Omnivores	Carnivores	Herbi-detritivores
SOM	0.10 (0-0.37)	0.09 (0-0.33)	0.14 (0-0.47)
DOC	0.14 (0-0.49)	0.13 (0-0.44)	0.18 (0-0.63)
<i>Sphagnum</i>	0.09 (0-0.33)	0.08 (0-0.29)	0.12 (0-0.42)
Vascular plants	0.11 (0-0.38)	0.10 (0-0.34)	0.14 (0-0.49)
Periphyton	0.49 (0.37-0.60)	0.55 (0.44-0.65)	0.34 (0.18-0.49)
Green algae	0.07 (0-0.24)	0.06 (0-0.21)	0.09 (0-0.30)

Table 5. PLFA composition, as percentage of total PLFAs, in sedimented organic matter (SOM) and aquatic invertebrates.

PLFA	SOM	Zoo-plankton	Zygotera nymphs <i>Enallagma</i>	Lestes <i>Leucorrhinia</i>	Anisoptera nymphs <i>Leucorrhinia</i>	Albifrons <i>albifrons</i>	Aeshna <i>juncia</i>	Corixa <i>dentipes</i>	Adult Heteroptera <i>Cymatia</i>	Notonecta <i>lutea</i>	<i>Ilybius</i> <i>genescens</i>	<i>Ilybius</i> <i>guttiger</i>	Laccophilus <i>poecilus</i>	Midge larvae <i>Chaoborus</i> spec.
Methane Oxidizing Bacteria Type 1														
16:1ω8c	0.5		0.0	0.0	0.1	0.2	0.2	0.2	0.1	0.1	0.0	0.1	0.1	
16:1ω5t	0.5	1.1	0.2	0.2	0.3	0.2	0.7	0.7	0.6	0.3	0.3	0.5	0.4	0.6
Total	1.1	1.1	0.2	0.3	0.4	0.4	0.9	0.9	0.7	0.4	0.3	0.6	0.5	0.6
Methane Oxidizing Bacteria Type 2														
18:1ω8c	2.6													
Methyl-branched														
10Me16:0	1.9											0.0		
10Me17:0	0.3	0.2			0.2									
10Me18:0	0.6		0.2			0.1	0.1	0.1	0.1	0.1			0.1	0.3
Total	2.8	0.2	0.2	0.0	0.2	0.1	0.1	0.1	0.0	0.1	0.0	0.0	0.1	0.3
Branched unsaturated														
i17:1ω7c	0.2	0.4												
Branched saturated														
i14:0	0.7	0.1			0.2				0.1					
i15:0	3.9	0.3	0.2		0.1		0.2	0.2	0.2				0.2	0.2
a15:0	4.1	0.2												
i16:0	1.5	0.2	0.1		0.2	0.2	0.2	0.2	0.5	0.4		0.1	0.3	0.3
a17:0	1.1	0.3	0.1	0.1	0.2	0.2	0.4	0.4	0.4	0.1		0.1	0.2	0.4
br17:0	0.2	0.1					0.1	0.1	0.1	0.2		0.1	0.1	0.2
Total	11.6	1.2	0.4	0.1	0.5	0.4	0.9	0.9	1.4	0.7	0.0	0.4	0.8	1.0
With cyclopropyl rings														
cy17:0		1.1		0.4			0.4			1.0	0.4	0.6		0.5
cy19:0	7.8	0.2	1.1		0.9	0.8	1.4	1.4	0.7	0.6	0.3	0.5	0.4	0.5
Total	7.8	1.3	1.1	0.4	0.9	0.8	1.8	1.8	0.7	1.6	0.7	1.1	0.4	1.0
Polyunsaturated														
18:2ω6c,9c			0.2				0.1			18.2	24.5	21.4	0.1	
18:2ω6c,12c	0.4													
18:2ω7c,12c	0.5				0.2		0.1							
18:3ω4	1.9	0.2			0.3									
18:3ω6	0.3		9.9	11.4	10.7	6.9	7.4	7.4	7.2	4.7	10.3	7.9	4.0	10.0
20:4ω?	0.8	1.2	14.4	9.2	14.7	18.5	13.5	13.5	20.9	13.2	9.3	12.1	24.0	7.4
20:5ω3	0.6	3.9	14.3	16.8	13.6	10.9	20.2	20.2	16.8	11.3	8.4	9.7	14.1	22.6

Total	4.5	5.2	38.8	37.3	39.5	36.3	41.4	44.9	47.4	52.6	51.2	42.2	39.9
Monounsaturated													
16:1 ω 9t		0.1					0.1	0.3				0.1	0.2
16:1 ω 9c	0.6				0.6	0.1							
16:1 ω 7c	4.2	10.0	1.6	1.2	2.0	1.8	3.6	2.8	2.3	1.7	2.4	5.9	2.2
16:1 ω 6c	0.7		0.2	0.2	0.2	0.3	0.4	0.4	0.1		0.2	0.3	0.3
16:1 ω 5c	0.6	0.2	0.1		0.1	0.2	0.2				0.1	0.2	0.2
17:1 ω 6c	0.4	0.3	0.1		0.1	0.2	0.2	0.4	0.3			0.2	0.1
18:1 ω 11t	0.9	0.3			0.2			0.3					
18:1 ω 9c	5.3	10.6	20.6	21.6	21.8	23.5	21.3	17.3	23.6	24.3	24.6	20.1	18.4
18:1 ω 9t	1.6	0.1			0.2								
18:1 ω 7c	31.0	7.2	8.6	8.3	6.8	8.6	6.2	5.7	2.3	1.3	2.4	3.8	4.6
18:1 ω 5c	0.2				0.1	0.2	0.1						
20:1 ω ?	0.3	0.3		0.3	0.4	0.2	0.2	0.2	0.1	0.3	0.2	0.2	0.2
Total	45.7	29.2	31.2	31.6	32.6	35.2	32.3	27.3	28.7	27.6	29.9	30.7	26.1
Saturated													
14:0	2.0	6.6	0.2	0.1	0.3	0.2	0.3	0.4	0.2	0.1	0.3	0.4	0.9
15:0	0.5	2.3	0.3	0.3	0.3	0.3	0.3	0.3	0.2		0.2	0.3	0.6
16:0	14.4	41.4	14.5	17.6	11.6	10.7	9.4	8.8	11.2	12.8	10.9	13.1	21.0
17:0	0.8	1.5	0.2	0.2	0.2	0.3	0.3	1.8			0.1	0.2	0.4
18:0	3.9	8.2	11.7	10.4	12.9	14.0	12.1	12.5	8.3	5.3	4.8	10.2	7.6
20:0	0.2	0.4	0.2										
22:0	1.4	0.2											
Total	23.2	60.6	27.1	28.6	25.2	25.4	22.2	23.7	19.9	18.2	16.3	24.2	30.5
Estimated chain length													
ECL17.344	0.3		0.5		0.5	0.8		0.7				0.6	
ECL17.311	0.6				0.7								
ECL17.392	0.1	0.1			0.3								
ECL17.488								0.1	0.5				
ECL17.844									0.2				
Total	1.0	0.1	0.5	0.0	1.4	0.8	0.0	0.8	0.7	0.0	0.0	0.6	0.0
Bacterial total*	57.1	11.4	10.4	9.2	8.8	10.3	9.9	8.4	5.0	2.3	4.4	5.6	7.5

* Bacterial total gives the sum of the relative amounts of the PLFAs characteristic for MOB type 1 and 2, 18:1 ω 7c, the branched PLFAs and the PLFAs with cyclopropyl rings. The ? in 20:4 ω ? means that the position of the double bond nearest to the aliphatic end of the molecule was not identified.

Table 6. $\delta^{13}\text{C}$ (‰) of PLFAs in sedimented organic matter (SOM) and aquatic invertebrates.

PLFA	SOM	Zoo-plankton	Zygoptera larvae			Anisoptera larvae			Adult Heteroptera			Adult Coleoptera			Midge larvae
			<i>Enallagma cyathigerum</i>	<i>Lestes sponsa</i>	<i>Lestes sponsa</i>	<i>Leucorrhinia albifrons</i>	<i>Aeshna juncea</i>	<i>Corixa dentipes</i>	<i>Cymatia bonndorffii</i>	<i>Notonecta lutea</i>	<i>Ilybius aenescens</i>	<i>Ilybius guttiger</i>	<i>Laccophilus poecilus</i>	<i>Chaoborus spec.</i>	
14:0	-32.6	-37.4													
i15:0	-35.5														
a15:0	-33.8														
15:0		-29.5													
i16:0	-29.9														
16:0	-32.6	-36.3	-33.4	-34.8	-34.8	-33.4	-33.3	-34.1	-34.8	-32.5	-32.0	-32.8	-32.9	-36.9	
16:1w7c	-36.7	-27.9	-31.3	-32.7	-32.7	-32.1	-37.2	-40.4	-31.2	-33.0	-32.7	-33.8	-33.4	-37.6	
10Me16:0	-34.4														
a17:0	-31.4														
17:0	-35.1	-41.4							-34.1						
cy17:0	-38.5									-35.6					
18:0	-31.0	-38.0	-32.5	-34.8	-34.8	-33.2	-32.1	-33.8	-34.1	-32.3	-30.8	-31.7	-32.4	-38.1	
18:1w11t	-35.5														
18:1w9c	-32.5	-30.6	-33.3	-34.1	-34.1	-33.4	-32.5	-34.4	-34.5	-32.5	-31.3	-32.8	-32.8	-36.4	
18:1w9t	-35.6														
18:1w8c	-38.0														
18:1w7c	-35.3	-30.6	-33.2	-33.2	-33.2	-33.5	-31.7	-35.9	-31.6	-33.5	-32.9	-29.8	-33.7	-34.4	
18:2w6c,9c										-32.7	-32.9	-33.1			
18:3w6			-40.5	-41.4	-41.4	-37.5	-39.5	-37.2	-42.3	-37.5	-39.2	-40.6	-40.3	-40.9	
18:3w4	-34.2														
cy19:0	-35.6		-32.3					-37.6							
20:4w?	-45.5	-47.1	-33.6	-39.3	-39.3	-33.2	-34.2	-36.7	-36.5	-34.0	-34.2	-35.4	-34.5	-40.3	
20:5w3	-30.6	-34.5	-37.1	-37.2	-37.2	-36.0	-34.0	-32.1	-39.2	-35.3	-34.8	-37.4	-37.2	-36.2	

Nichols et al. 1985, Bowman et al. 1993) were found in SOM and all invertebrates (Table 5), but at low concentrations. The $\delta^{13}\text{C}$ values of these PLFAs could not be measured because of these low concentrations (about 1% of the total amount of PLFA or less). The PLFA 18:1 ω 8c, characteristic for MOB II (Bodelier et al. 2009), was found in SOM and had a $\delta^{13}\text{C}$ value of -38.0‰ (Table 6). This PLFA was not detected in the invertebrates. Here, it must be noted that in the PLFA analysis of the invertebrates the high peak of the PLFA 18:1 ω 9c may have hidden the peak of the PLFA 18:1 ω 8c (Deines et al. 2007). The PLFA 18:1 ω 9c was present in high concentration in all invertebrates and in a lower concentration in SOM. In addition, PLFAs 18:2 ω 6c,12c and 18:2 ω 7c,12c, that are diagnostic biomarkers of *Methylocystis* strains (MOB II) according to Bodelier et al. (2009), were detected in SOM and two insect species.

Methyl-branched and branched unsaturated PLFAs that are typical for sulphate-reducing bacteria and actinomycetes (Kroppenstedt 1992, O'Leary & Wilkinson 1988) were found at low concentrations in SOM and occasionally in invertebrates. PLFAs with cyclopropyl rings and branched PLFAs that are typical for bacteria (O'Leary & Wilkinson 1988, Zelles 1999) had a substantial concentration in SOM, but were found in low concentrations in the invertebrates. The $\delta^{13}\text{C}$ values of these PLFAs varied between -38.5 and -29.9‰. The monounsaturated PLFA 18:1 ω 7c, typical for bacteria (Wilkinson 1988) and a major PLFA in MOB (Bodelier et al. 2009), but also found in low abundance in various groups of algae (Dijkman & Kromkamp 2006), was the most abundant PLFA in SOM and present in all invertebrates and had $\delta^{13}\text{C}$ values between -35.9 and -29.8‰. The total relative concentration of the above mentioned PLFAs typical for bacteria was 57.1% in SOM, 11.4% in zooplankton, and between 2.3% and 10.4% in the insect species.

The total relative concentration of polyunsaturated PLFAs (PUFAs) was 4.5% in SOM and 5.2% in zooplankton, but between 36.3% and 52.6% in the insects. The PLFAs 18:3 ω 6, 20:4 ω ?, and 20:5 ω 3 were found in considerable amounts in all insects analysed here. The PLFA 18:2 ω 6c,9c was present in high concentration only in the predators *Notonecta lutea*, *Ilybius aenescens* and *Ilybius guttiger* and (almost) absent in the other invertebrates and SOM. The PLFA 18:3 ω 6 is reported from algae (Chrysophyceae) and Cyanobacteria (Taipale et al. 2009 and references therein) and fungi (Desvillettes et al. 1997). The PLFA 20:4 ω 6 is found in minor amounts in some Bacillariophyceae (diatoms), but in higher abundance in Rhodophyta (Dijkman and Kromkamp 2006). The PLFAs 20:4 ω 6c and 18:3 ω 6c are both produced by Protozoans grazing on MOB in wet soils (Murase et al. 2011). The PLFA 20:5 ω 3 is typical for various groups of algae, including diatoms and Cryptophyta (Dijkman & Kromkamp 2006). The PLFA 18:2 ω 6 is used as biomarker for fungi (Frostegård & Bååth 1996, Desvillettes et al. 1997), for plant detritus in a freshwater system (Jaschinski et al. 2011) and also present in considerable amounts in algae species of the Chlorophyta group (Dijkman & Kromkamp 2006) and in Cyanobacteria (Caramujo et al. 2008).

PUFAs had the most depleted $\delta^{13}\text{C}$ values among all PLFAs found. The PUFA 20:4 was the most depleted PLFA in SOM and zooplankton with $\delta^{13}\text{C}$ values of -45.5‰ and -47.1‰, respectively, but less depleted in the insects, with $\delta^{13}\text{C}$ values between -40.3 and -33.2‰. In contrast to the PLFA 20:4, the PLFA 20:5 ω 3 was more enriched in ^{13}C in SOM than in the invertebrates. In most insects the PUFA 18:3 ω 6 was the most depleted PLFA with $\delta^{13}\text{C}$ values between -42.3 and -37.2‰. This PUFA was not detected in zooplankton and had a low concentration in SOM. Therefore no reliable $\delta^{13}\text{C}$ value could be obtained for 18:3 ω 6 in SOM.

Among the monounsaturated PLFAs (MUFAs), 18:1 ω 9c had the highest relative concentration in the insect species (between 17.3% and 24.6% of the total amount of PLFAs). In seston this PLFA is used as an indication for the phytoplankton group Chlorophyceae, in particular *Chlamydomonas* sp. (Taipale et al. 2009), but occurs in other algae (Dijkman & Kromkamp 2006) and Cyanobacteria (Caramujo et al. 2008) as well. The high relative concentration of 18:1 ω 9c in the insects indicates the ingestion of algae by these insects or by their prey. However, 18:1 ω 9 is also a major PLFA of methanotrophs in wet peat soils (Chen et al. 2008). In zooplankton the relative concentration of 18:1 ω 9c was with 10.6% lower than in the insects, but also the highest among the MUFAs, followed by 16:1 ω 7c (10.0%) and 18:1 ω 7c (7.2%). The PLFA 16:1 ω 7c is a major PLFA in methanotrophic bacteria, but also in green sulphur bacteria (Taipale et al. 2009 and references therein), nitrifiers (De Bie et al. 2002), and diatoms (Dijkman & Kromkamp 2006).

The total relative concentration of saturated PLFAs was between 16.3% and 30.5% in SOM and insects, but 60.6% in zooplankton, with 41.4% consisting of the PLFA 16:0. This PLFA is also abundant in several groups of algae and bacteria (Taipale et al. 2009 and references therein).

6.4 Discussion

Stable isotopic signatures and the role of periphyton and other potential food sources

The stable isotopic signatures of the aquatic invertebrates and the living and dead tissue of primary producers in the raised bog pools showed the presence of multiple trophic levels (Figure 2) and a differential use of basal food sources by the invertebrates, not only between species, but also among individuals, as well as different size classes of the same taxa (Table 2). The dominant primary producers in these pools (*Sphagnum* mosses and vascular plants) and their dead sedimented organic matter (SOM) can potentially sustain the less depleted half of the invertebrate food web with $\delta^{13}\text{C}$ values $> -28\text{‰}$. The $\delta^{13}\text{C}$ values of dissolved organic substances (DOC) and invertebrates walking and flying around the bog pools were in the same range. Periphyton, predominantly consisting of algae, but likely containing different kinds of microbes as well, is the only potential food source found sufficiently depleted in ^{13}C to sustain at least the half of the invertebrate food web which is more depleted in ^{13}C . The $\delta^{13}\text{C}$ values of periphyton varied considerably between the pools. In pool N1 it had a $\delta^{13}\text{C}$ value of -27.4‰ . Possibly, periphyton, or periphyton components, with a $\delta^{13}\text{C}$ value $< -30\text{‰}$ were also present in N1, like in N2 and N3. In addition, the $\delta^{13}\text{C}$ values of phytoplankton can be lower than -30‰ (Taipale et al. 2007). The variation in $\delta^{13}\text{C}$ values of the different samples of periphyton and larger algae collected in this study (between -34.7‰ and -19.4‰ ; Table 2) and the variation in $\delta^{13}\text{C}$ values of algae during the year and between algae species found in other studies (e.g. Bontes et al. 2006) is high. Sampling of periphyton, as well as phytoplankton, in the bog pools at different moments in the same year as the invertebrates would have given more detailed information about the variation in the $\delta^{13}\text{C}$ values of these potential food sources. It is likely that these values varied during the year and that algae in periphyton and possibly also phytoplankton could be the basal food source sustaining the more depleted half of the invertebrate food web in all three bog pools sampled here.

The stable isotopic signatures of the invertebrates and potential basal food sources alone do not resolve the importance of living or dead material of *Sphagnum* mosses and vascular plants, or particulate and dissolved organic matter originating from the peat in which the pools are

embedded, as basal food source for the invertebrates with $\delta^{13}\text{C}$ values $> -28\%$. The range in $\delta^{13}\text{C}$ values of the algae samples collected here (periphyton and larger algae) implies that the whole invertebrate community could be sustained only by different species of algae. Alternatively, the carbon sources for the invertebrates could consist of a combination of algae, living or dead organic material from macrophytes, dissolved organic carbon compounds and bacteria and fungi living in or on the various organic substrates.

Biomarker PLFAs and pathways in the food web

The variation in the PLFA composition (Table 4) and in the $\delta^{13}\text{C}$ values of PLFAs (Table 5) of the invertebrates indicates that they used different basal food sources. They assimilated fatty acids that are specific for MOB, for other bacteria, as well as polyunsaturated fatty acids (PUFAs) that are derived from algae, and maybe macrophytes, either or not via fungi. These PLFA data confirm the importance of algae (periphyton and possibly phytoplankton) inferred from the stable isotope data. The elucidation of the relative importance of these basal food sources to the invertebrate food web of bog pools is however somewhat constrained because many of the recorded PLFAs cannot unambiguously be attributed to either macrophytes, algae, or methanotrophic or other bacteria. Furthermore, eukaryotes other than algae or macrophytes might synthesize PUFAs that are used as biomarkers for algae.

The PUFAs 20:5 ω 3, assumed to be characteristic for algae, as well as 20:4 ω 6, can be produced by zooplankton from 18:3 ω 3 and 18:2 ω 6, respectively, although this ability differs between groups (Caramujo et al. 2008 and references therein). According to Arts (1999) most species of freshwater zooplankton cannot synthesize or elongate PUFAs with 18 or 20 C-atoms and must obtain them from their diet. This inability is also found for omnivorous caddisfly larvae and the PUFAs 18:2 ω 6, 18:3 ω 3, 20:4 ω 6, and 20:5 ω 3 (Torres-Ruiz et al. 2010). This finding may therefore be extended for the invertebrate species studied here. Murase et al. (2010) found that the PLFAs 18:3 ω 6 and 20:4 ω 6 were produced by protozoans grazing on MOB. It is unknown if this pathway is important in bog pools. However, it is unlikely to be the only pathway conveying these PLFAs to the insects, as the amounts of 18:3 ω 6, 20:4 ω ?, 20:5 ω 3, and their possible ω 6 and ω 3 precursors in SOM and zooplankton are small compared to the high amounts of these PUFAs in the insects (Table 5), although preferential assimilation of PUFAs by the insects might result in a higher relative amount of PUFAs in the insects than in their food. Moreover, the abundance of green algae and the presence of some pieces of *Sphagnum* mosses and other macrophytes in the guts of several insects in the pools studied here (Odonata nymphs and larvae of Chironomidae and Trichoptera; personal observations) support the conclusion that algae are indeed an important basal food source in the bog pool food web, next to bacteria and possibly macrophytes, especially for the insects that all showed a high concentration of PUFAs (Figure 3).

The amount of 20:5 ω 3, characteristic for diatoms and also present in some other groups of algae, but absent in bacteria, might help to roughly estimate the relative amount of PLFAs originating from either bacteria or algae in SOM and invertebrates, when a more or less constant ratio between 20:5 ω 3 and the other PLFAs in the algal community is assumed. The relative amount of the PLFA 20:5 ω 3 in zooplankton was six times higher than in SOM, and in insects it was fourteen to thirty-seven times higher than in SOM (Table 5). Thus, most of the PLFA content in SOM was present in the bacterial community, and possibly partly in protozoa like flagellates and ciliates that ingested them. In the invertebrates much larger parts of the PLFA content originated from algae, but the insects seem to rely more on algae than pelagic zooplankton does (Figure 3).

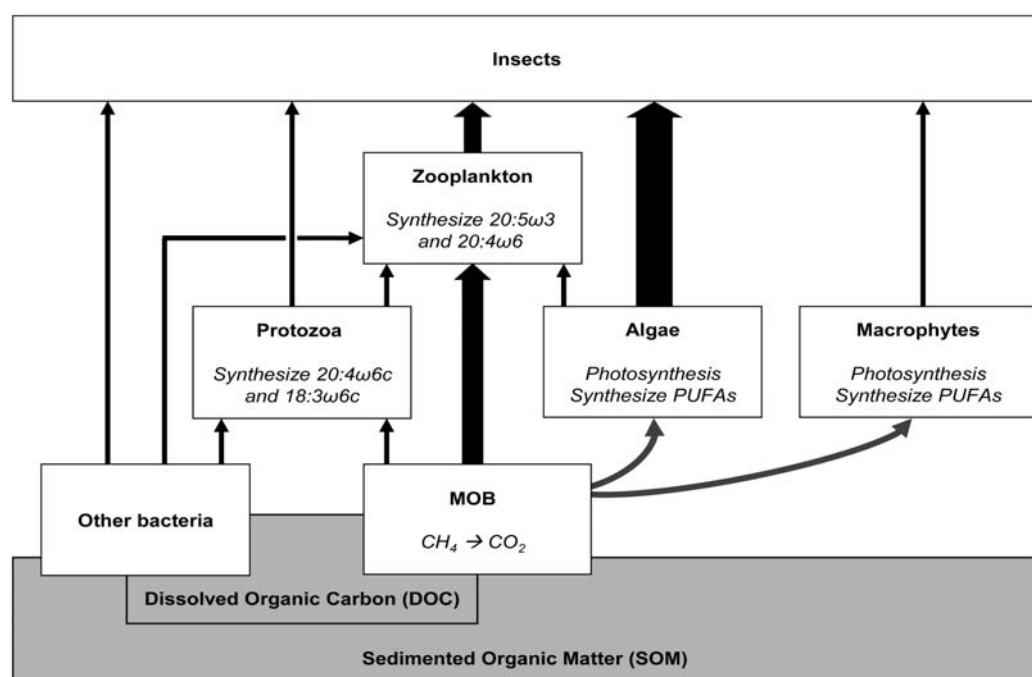


Figure 3. Schematic representation of the food web in raised bog pools. The thickness of the black arrows indicate the relative importance of that relation to the invertebrate group, as derived from the results described in this paper. The grey curved arrows indicate the possible role of methane oxidising bacteria (MOB) in the carbon supply to the primary producers.

In the zooplankton the total relative amount of PLFAs characteristic for bacteria given in Table 5 (11.4%) was much lower than in SOM (57.1%) and closer to the range found in the insects (2.3–10.4%). However, PLFA composition was different, with a high amount of the PLFAs 14:0, 16:0 and 16:1ω7c in the zooplankton relative to both SOM and insects. Remarkably, these were the main PLFAs in which labelled methane was incorporated in forest soil samples (Knief et al. 2003). The relative amount of the PLFA 16:1ω5t, typical for MOB I, was also higher in the zooplankton than in SOM and insects. Taken together, this suggests that the zooplankton assimilated much more fatty acids originating from MOB (ingested directly as part of ingested seston or via protozoans) and two to six times less from algae than the insects did, directly or via their prey (Figure 3). Additionally, differences between SOM and zooplankton in their PLFA composition and in the $\delta^{13}\text{C}$ values of PLFAs can result from preferential assimilation of PLFAs by zooplankton. Preferential ingestion of bacteria by the protozoa upon which the zooplankton preys is another possibility. For example, Murase and colleagues (2010) found protozoans preferring MOB I above MOB II. Finally, the composition of the bacteria community may differ between the seston ingested by zooplankton (collected in open water) and SOM (collected at the bottom of the pool), with a higher relative abundance of MOB in the seston. Elucidation of the various pathways in which this could come about requires further investigations, but the available data indicate that MOB are a significant food source for pelagic zooplankton in bog pools (Figure 3), something which was also found in lake pelagic food webs (Bastviken et al. 2003, Taipale et al. 2007).

The PLFA composition of the collected SOM shows that the living biomass in SOM is dominated by bacteria. In a wide range of bacterial dominated sediments the sum of the relative amounts of the PLFAs i14:0, a15:0, i15:0, i16:0, and 18:1 ω 7c, characteristic for bacteria, is $28\pm 4\%$ (Middelburg et al. 2000). This sum was much higher in the SOM of the bog pools studied here (41.3%), due to the high abundance of 18:1 ω 7c (31%). The PLFA 18:1 ω 7c is likely to be the prevailing lipid in methanotrophs in *Sphagnum* moss (Bodelier et al. 2009, Van Winden et al. 2010). Using the relative amount of the MOB specific PLFAs 16:1 ω 8c and 18:1 ω 8c in SOM and the fairly constant ratio between these specific PLFAs and non-specific PLFAs found in MOB strains (Bodelier et al. 2009), we may assume the MOB to make up about 10% of the bacterial population in the SOM.

A pathway of methane to invertebrates via algae?

As methane and MOB are depleted in ^{13}C , the $\delta^{13}\text{C}$ values of invertebrates assimilating methane-derived carbon are similarly depleted (Taipale et al. 2007 and 2009). The low $\delta^{13}\text{C}$ values of the zooplankton samples compared to most insects, including all insects of low trophic level (Table 2), indeed corresponds to the larger reliance of zooplankton on MOB inferred from the PLFA data. However, overall, the PUFAs characteristic for algae or other plants (18:3 ω 6, 20:4 ω ?, 20:5 ω 3) were more depleted than the PLFAs typical for bacteria, including the PLFAs typical for MOB. For some of these PUFAs this could be explained by the possibility that they can also be synthesized by protozoans (c.f. Murase et al. 2010) or zooplankton (c.f. Caramujo et al. 2008). The PLFA 20:4 ω ? was much more depleted in ^{13}C in the zooplankton than in the insects, indicating a difference in carbon pathways. As methane is known to be depleted in ^{13}C , this would suggest that the zooplankton synthesized this PLFA from precursor fatty acids (c.f. Caramujo et al. 2008) ingested via MOB, or that they ingested protozoa that synthesized this PLFA (c.f. Murase et al. 2010), whereas the insects might get the PLFA 20:4 ω ? via algae and herbivorous prey.

It is, however, remarkable that in zooplankton the $\delta^{13}\text{C}$ values of the PLFAs 16:1 ω 7c, presumably derived from MOB, as suggested above, and 18:1 ω 7c, derived from MOB and other bacteria, were also generally less depleted than in the insects (Table 6). As methane is depleted in ^{13}C , the $\delta^{13}\text{C}$ values of the PLFAs in MOB are expected to be lower than those in algae, assuming the latter use CO_2 for photosynthesis. However, Raghoebarsing et al. (2005) showed that submerged *Sphagnum* mosses can use CH_4 as carbon source, converted to CO_2 via endosymbiotic MOB. Could also the algae in bog pools obtain methane-derived CO_2 via MOB living as endosymbionts or as a constituent of the periphyton (or biofilm), explaining the relatively depleted $\delta^{13}\text{C}$ values of algae-derived PUFAs in the insects? Labelling studies with ^{13}C -bicarbonate or $^{13}\text{CH}_4$ (Raghoebarsing et al. 2005, Deines et al. 2007, Pace et al. 2007) are required to verify the existence of such intriguing pathways from both MOB and algae in the food web of bog pools.

Combining the outcomes of stable isotopes and PLFA analyses

The variation in the stable isotopic signatures (Table 2), the PLFA composition (Table 5), and the $\delta^{13}\text{C}$ values of PLFAs (Table 6) indicated that the invertebrates in bog pools use different basal food sources. The $\delta^{13}\text{C}$ values of different potential basal food sources and invertebrates indicated that algae (in periphyton and possibly phytoplankton) sustain at least half the invertebrate food web. The PLFA composition showed that algae, MOB and other bacteria are ingested by the invertebrates, directly or via their prey. Pelagic zooplankton seems to rely more on bacteria, whereas for insects algae are more important. This variation in relative importance of basal food sources is indicated in the schematic representation of the food web (Figure 3) with variation

in the thickness of the black arrows. A considerable part of the bacterial biomass conveyed to higher trophic levels in the bog pools likely originates from MOB. The results suggest that algae in bog pools use methane derived carbon, possibly via MOB (indicated with the grey curved arrows in Figure 3). Invertebrates grazing on periphyton likely ingest the MOB associated with the periphyton. Thus, depleted $\delta^{13}\text{C}$ values of whole organisms, or PLFAs, do not necessarily implicate a direct pathway between MOB and these organisms. Instead, algae could be an intermediate, constituting a major food source for aquatic invertebrates.

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Chapter 7

Synthesis

Gert-Jan van Duinen

◀ The aquatic beetle *Laccophilus poecilus* is common in pristine raised bog pools in Estonia, but absent from most bog remnants in The Netherlands. (Photo: René Krekels)

7.1 Introduction

Raised bog landscapes have been severely degraded or completely reclaimed for agriculture, afforestation, and peat extraction in more densely populated parts of their distribution area. Remaining intact and degraded bogs are currently under pressure of drainage, expanding infrastructure, the need for peat for horticultural purposes and energy, and atmospheric pollution (Joosten & Clarke 2002). Restoration measures are carried out in raised bog remnants abandoned after peat extraction or forestry. These measures include rainwater retention, aiming at regenerating a self-sustaining bog ecosystem or maintaining viable populations of characteristic species (Wheeler & Shaw 1995, Vasander et al. 2003, Rochefort & Lode 2006). Effects of both degradation and restoration measures on hydrology, vegetation and carbon cycling in raised bogs have been intensively studied over the past decades (e.g. Schouten 2002, Price et al. 2003, Holden et al. 2004, Couwenberg et al. 2011), but invertebrate fauna remained largely unstudied (Wheeler & Shaw 1995, Schouwenaars et al. 2002). In this thesis the effectiveness of bog restoration measures for rehabilitation of aquatic invertebrate communities and key factors involved were assessed using data of comparative field surveys in:

- A. Natural water bodies in different parts of pristine raised bog landscapes in Estonia,
- B. Water bodies that remained after ‘traditional’ exploitation of raised bogs in The Netherlands abandoned before 1950 (small-scale hand peat cutting pits and trenches; called remnant sites), and
- C. Water bodies created by rewetting of bog remnants in The Netherlands with different baseline situations (small-scale traditional and large-scale industrial exploitation) and recovery periods varying between 1-30 years since rewetting (called restoration sites).

This chapter addresses the central thesis aim and main research questions by combining and discussing results of the research presented in the previous chapters and other relevant studies. First, the effects of degradation on aquatic invertebrate communities in raised bog landscapes are discussed. Section 7.2 addresses the loss of habitat diversity present in pristine raised bog landscapes, whereas effects of increased nutrient availability on the invertebrate community and food web are discussed in section 7.3. Next, section 7.4 addresses the effectiveness of current restoration measures in the rehabilitation of aquatic invertebrate communities in raised bog landscapes and factors, which facilitate or limit the rehabilitation of aquatic invertebrate communities. Persistence of species and re-colonisation are discussed in section 7.5.

Local species assemblages are often considered as subsets of the regional species pool delimited by a set of abiotic and biotic filters (Poff 1997, Weiher et al. 2011). Changes in environmental conditions in raised bog landscapes caused by both degradation and restoration may be viewed as filters acting on the aquatic invertebrate community. Section 7.6 summarizes how degradation and restoration measures act as filters on the aquatic invertebrate community of raised bog landscapes. This chapter concludes with answering the three main research questions formulated in the introduction of this thesis (section 7.7) and recommendations for restoration practice of raised bogs and further research (sections 7.8 and 7.9).

In Chapter 1 a schematic presentation was given of the past and current human activities that impact the environmental conditions and mechanisms structuring the invertebrate community of raised bog landscapes. This scheme is repeated here (Figure 4), but with the different blocks presenting a summary of the changes in environmental conditions, the invertebrates’ responses on these changes and the corresponding changes in indices of the invertebrate community assessed

in this thesis. The lower part of the scheme presents the conclusions and recommendations for restoration management and further research.

7.2 Effects of degradation of raised bog landscapes on aquatic invertebrate communities

The degradation of raised bog landscapes in The Netherlands has resulted in the loss of the environmental conditions and subsequently aquatic invertebrate communities present in the most nutrient-poor water bodies in pristine raised bog massifs, as well as in the lagg zone (Chapter 4 and 5). Many species that are abundant in Dutch raised bog remnants prefer transitional mires in Estonian raised bog landscapes, or are not present at all in intact Estonian bogs. The invasion of degraded bog massifs by species that do not occur in pristine ombrotrophic conditions is also assessed by investigations of other sites and taxonomic groups (Koponen 1979, Burmeister 1980, Wheeler & Shaw 1995). By assessing the environmental conditions and the occurrence of aquatic macroinvertebrate species in the different parts of pristine raised bog landscapes and in Dutch bog remnants the current macroinvertebrate composition in Dutch bog remnants could be attributed to either the loss of groundwater influenced parts of bog landscapes or nutrient enrichment (Chapter 4 and 5; section 7.3).

Groundwater influence is lacking in most Dutch raised bog remnants that are mostly remnants of ombrotrophic bog massifs and surrounded by intensively drained agricultural and urban area (Chapter 1). Consequently, several macroinvertebrate species that in Estonian raised bog landscapes only occur in transitional mires and lags are absent or rare in Dutch bog remnants (Chapter 4). Van Kleef et al. (2012) found that 21 of 33 species (including vascular plant, odonates, water beetles and caddis flies) endangered in The Netherlands and present in Estonian raised bogs were recorded more often in the transitional mire and lagg zone of Estonian bogs than in the bog massif. This stresses the importance of the different zones in complete raised bog landscapes as habitat for endangered species. Apparently, these 21 species as well as several other endangered species of taxonomic groups for which no Red List exists in The Netherlands do not profit from increased nutrient availability (see examples in Chapter 4). This response is opposite to species that also occur more frequently in the transitional mire and lagg zone of Estonian bogs than in the bog massif and that are more abundant in Dutch bog remnants than in Estonian bog landscapes. This differential response of species indicates that the aquatic macroinvertebrate communities of pristine transitional mires and lags consist of species that require a higher nutrient availability and species that require a higher availability of minerals or acid buffering provided by the buffered groundwater, irrespective of a higher nutrient availability. The first group of species have increased their abundance in Dutch bog remnants, whereas the second group decreased. For aquatic worms (oligochaetes) this differential response could be linked to differences in their diet (Chapter 5).

In the peat cutting pits and trenches that remained after ‘traditional’ exploitation of raised bogs a higher variation in species composition (dissimilarity) and a higher number of characteristic macroinvertebrate species was found than in water bodies created by large-scale rewetting measures (Chapter 2 and 4). This high dissimilarity can be attributed to the environmental heterogeneity resulting from natural and human-induced variation in environmental conditions in bog remnants (Verberk et al. 2006, 2010). The higher number of characteristic species can be explained by persistence of populations during the gradual process of degradation of bogs. Next to the gradual loss of suitable natural pools and puddles, man-made peat cutting pits and trenches provided new habitat enabling these species to survive as relict populations. Part of the

studied water bodies created by rewetting were remnants of industrial peat extraction where aquatic invertebrates could not survive the period of exploitation, whereas other restoration sites were created by inundation of mosaics of peat cutting pits and dry strips of peat.

7.3 Effects of increased nutrient availability on the invertebrate community

The nutrient availability in pristine ombrotrophic raised bog massifs naturally is extremely low. The increase in nutrient availability, resulting from increased atmospheric N and S deposition in bog remnants in industrialized and agricultural regions (Berendse et al. 2001, Roelofs 1986), as well as increased mineralization due to drainage (Tomassen et al. 2004), affects the aquatic invertebrate community. Chapter 4 reports a 4-6 fold increase of N and P compounds in surface water of Dutch bogs, compared to Estonian bogs. In The Netherlands the occurrence of invertebrate species in acid bog water bodies is not limited anymore by nutrient availability. The comparative study between Estonia and The Netherlands showed a higher total macroinvertebrate abundance in Dutch water bodies (Chapter 4). This increased abundance is mainly due to a number of species which apparently require higher nutrient availability, as indicated by their preference for transitional mires or laggs within pristine Estonian bog landscapes and high abundance in Dutch bog water bodies with low pH. For these species apparently not the higher pH or buffering capacity, but other conditions characteristic of transitional mires and laggs are important, most likely an increased resource availability. Suitable conditions for these species are in pristine raised bog landscapes only present if the decomposition rate of organic matter is high. The latter is a result of a lower C:P quotient of dead organic matter and a higher pH and buffering capacity of surface or interstitial water (Smolders et al. 2002), which are in transitional mires and laggs due to the influence of minerotrophic groundwater (Chapter 5).

In The Netherlands, these species appeared to be able to live in water bodies with a higher C:P quotient of organic matter and low pH. For herbivorous or detritivorous invertebrates, like the oligochaete *Nais variabilis* (Chapter 5), it may therefore be concluded that they respond to the abundance and nutritional value of diatoms or other algae, which are influenced by N and P availability in surface water, or a change in the quality of dead organic plant material. In the Dutch bog remnants the prolonged high deposition rates of N and S and the increase in primary production of algae and vascular plants, including the invasive grass species *Molinia caerulea*, may have led to a new type of dead organic matter that is more easily decomposed by microbes, although the bulk organic matter C:P quotient may still be relatively high. Several detritivorous and herbivorous invertebrate species may profit from the increased abundance of bacteria and/or (micro)algae resulting from the increased availability of nutrients (N and P) in Dutch bog remnants and consequently predator species may profit from the increased prey availability. However, the increased availability of N and P likely constrains other invertebrate species. These species may be negatively effected by excess dietary nutrient content (Nijssen & Siepel 2010, Elser et al. 2012, Turlure et al. 2013) or lose their competitive edge when nutrient availability increases (sensu Tessier & Woodruff 2002, Iwabuchi & Urabe 2012). In raised bog remnants, nutrient enrichment has created a novel environment with acid and nutrient rich conditions, absent in natural non-polluted ecosystems.

To increase our understanding of the structure and functioning of aquatic food webs and invertebrate communities in raised bog pools and the impact of increased nutrient availability, the basis of the food web in three pristine bog pools in Estonia was assessed by means of stable isotope ratios and PLFA composition (Chapter 6). This investigation revealed that algae - most

likely periphyton - are a principal basal food source for the invertebrate community. In addition, bacterial biomass is conveyed to higher trophic levels, with a considerable part likely originating from methane oxidizing bacteria (MOB). This study also points to the ingestion of algae that possibly obtained carbon from MOB. The incorporation of methane-derived carbon was previously found for submerged *Sphagnum* with endosymbiotic MOB (Raghoebarshing et al. 2005). Therefore, depleted stable carbon isotope ratios ($\delta^{13}\text{C}$) of invertebrates do not necessarily imply a direct pathway between MOB and these invertebrates. It may also reflect algal food sources forming an intermediate level.

The ingestion of MOB and algae that incorporated methane-derived carbon can explain the discrepancy between the $\delta^{13}\text{C}$ of the dominant primary producers (*Sphagnum* and vascular plants) and the more depleted $\delta^{13}\text{C}$ of many of the sampled macroinvertebrates and microinvertebrates (Chapter 6). In addition to the three Estonian bog pools, Van Duinen et al. (2006) also sampled six water bodies in Dutch bog remnants, differing in nutrient concentration. The discrepancy between the average $\delta^{13}\text{C}$ of dead organic matter and living plants and the average $\delta^{13}\text{C}$ of the invertebrates (hereafter termed average trophic shift) was not consistent for all these nine sampling sites. For the two most nutrient rich sampling sites all collected fauna species appeared to be similar or enriched in $\delta^{13}\text{C}$ compared to dead organic matter and living plants. The average trophic shift strongly and positively correlated to inorganic N and P concentrations of surface water and interstitial water.

Although the role of methane and periphyton -and possibly phytoplankton- can explain the negative average trophic shift in bog pools with low nutrient concentrations, it does not explain the absence of a negative average trophic shift in bog pools with high nutrient concentrations. This may suggest that with increasing nutrient availability dead organic matter and other types of algae (e.g., filamentous algae with less depleted $\delta^{13}\text{C}$ in pristine bog pools; Chapter 6) are of sufficient quality to support an increasing proportion of the invertebrate community. Concurrently, the role of methane and methanotrophic bacteria at the basis of the food web may decrease. In addition, the production of methane in the peat substrate decreases with a decrease in decomposability of the peat (Smolders et al. 2002), a decrease in the buffered groundwater influence, and an increase in sulphate concentration (Lamers et al. 1999). All of these factors may play a role in cut-over bogs in The Netherlands embedded in a deeply drained agricultural and urban landscape.

7.4 Effectiveness of raised bog restoration measures

Raised bog restoration measures focus on restoring growth conditions for the key stone genus *Sphagnum*, which is a prerequisite for the restoration of functioning of raised bog ecosystems (Rochefort 2000). Therefore, restoration projects include infilling or blocking of drainage ditches and the construction of bunds, intended to retain rainwater, to raise the water table to or above the surface of the remaining peat substrate, and to decrease fluctuations of the water table (Wheeler & Shaw 1995). Macroinvertebrate and microinvertebrate species, including characteristic and rare species, colonised large-scaled inundated cut-over peat fields (Chapter 2 and 3) where hardly any species could live prior to the rewetting measures. This should of course be judged as a positive result of these measures. Other studies showed the rapid colonisation of testate amoebae or flies (Brachycera) after recovery of a *Sphagnum*-dominated vegetation (Buttler et al. 1996, Taillefer & Wheeler 2012) and aquatic macroinvertebrates after digging of pools in a cut-over bog (Mazerolle et al. 2006).

Chapter 7

The baseline situation of the restoration sites sampled here differed considerably prior to rewetting. They included large-scale peat extraction fields as well as small-scale mosaics of old peat cutting pits and differed in peat quality and vegetation composition before the water level was raised by retention of rain water one to thirty years before sampling. After rewetting their macroinvertebrate species composition showed a high similarity (Chapter 2). This high similarity and lower cumulative species richness in the group of restoration sites compared to the remnant sites suggest that rewetting of mosaics with old peat cutting pits has likely had a negative impact on characteristic and rare species present until the restoration measures were carried out. Data of the macroinvertebrate species composition prior to rewetting of these sites were lacking. However, this conclusion corresponds well with the response of the aquatic macroinvertebrates to rewetting of a bog remnant where the species composition was assessed before and after large-scale restoration measures were carried out by which a loss of environmental heterogeneity and macroinvertebrate diversity occurred (Verberk et al. 2010). The responses of individual rare and characteristic species to rewetting assessed by Verberk et al. (2010) and found in our comparative study (Chapter 2) were highly similar. In total 14 out of 18 rare and characteristic species increased or decreased in occupancy in a similar way in both studies. The four exceptions were caused by occasional differences in site conditions or time lags (Verberk et al. 2010).

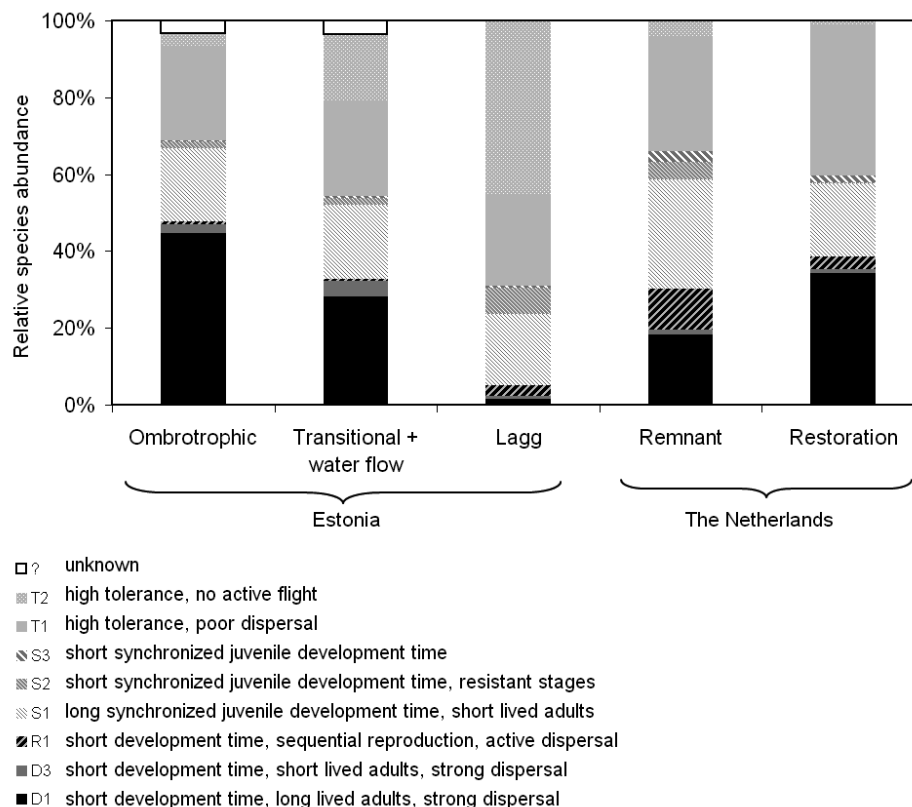


Figure 1. Average relative abundance of species classified in life history strategies according to Verberk et al. (2008) in ombrotrophic sites, transitional mire sites and those influenced by flowing water, and lags in pristine raised bogs in Estonia and remnant sites and restoration sites in The Netherlands.

The application of life-history strategies (see for classification the legends of Figure 1) in the analysis of species composition changes by Verberk et al. (2010) provided also understanding of the functional responses of species to environmental changes induced by rewetting measures. Retaining rain water in bog remnants may result in loss of groundwater influence locally present in bog remnants (Verberk et al. 2010). This reduces the predictability of environmental conditions. According to Verberk et al. (2010) rain water retention resulted in harsher and less predictable conditions, indicated by an increase in species tolerant for unfavourable conditions (life-history strategy T1) and a decrease in species with a strongly synchronised life cycle (S-strategies). These changes in life-history strategy composition were consistent with the differences in life-history strategy composition between remnant and restoration sites studied in this thesis (Figure 1).

The life-history strategy composition showed remarkable differences between the three groups of sites in pristine Estonian bogs, the remnant sites and the restoration sites. The relative abundance of species with a short development time and strong dispersal (D1) decreased, whereas species tolerant for unfavourable conditions (T1 and T2) increased when going from the ombrotrophic bog massif to the lagg zone. This increase of tolerant species was due to the increase of the abundance of oligochaetes with strategy T2. These species can tolerate long periods of harsh conditions (Verberk et al. 2008) and were very abundant in the lagg zone which are often dry in summer and may show periods of hypoxia. Species classified in strategy D1 showed the highest average relative abundance in ombrotrophic sites. These species may persist in raised bogs by exploiting short periods of suitable conditions in bog pools and oviposition at different locations. The relative abundance of D1-species in restoration sites was intermediate between remnant sites and ombrotrophic sites. The relative abundance of species with a strongly synchronized life cycle (mainly S1) was similar between the three groups of Estonian sites and the restoration sites, but was increased in the remnant sites. Nutrient enrichment was found to enable species to increase their abundance or to invade acid water bodies in Dutch bog remnants (Chapter 4, section 7.3), but may as well result in more frequent and intensive oxygen stress than in pristine ombrotrophic bog pools. This may explain the high abundance of T1-species in remnant and restoration sites.

The invertebrate species composition of the remnant sites included a similar amount of variation as the sites in intact bog landscapes (gradient in acidity, ombrotrophic to minerotrophic), whereas the species composition in restoration sites did not show similarity to those in water bodies influenced by buffered groundwater (Chapter 4). Similarly, the assessment of the effects of rewetting by Verberk et al. (2010) showed a decrease in the groundwater influence in part of the rewetted area resulting from retention of rain water in the area. Thus, until now raised bog restoration measures resulted in conservation or recolonisation of species characteristic of more ombrotrophic sites, but not in conservation or recolonisation of species characteristic of more minerotrophic sites. In the case of 'traditional' peat cuttings and persistence of relict populations as baseline situation retention of rain water may even cause loss of the species composition of the more minerotrophic part of the raised bog gradient. To conserve or restore the aquatic macroinvertebrate species diversity of raised bog landscapes (at macro-scale) the influence of groundwater should be preserved or restored.

Buffered groundwater also stimulates *Sphagnum* growth by floating raft formation, which is an option for restoration of *Sphagnum*-dominated vegetation on inundated cut-over peat substrate (Lamers et al. 1999, Smolders et al. 2002). If floating raft formation is not likely to occur after

inundation of cut-over peat due to improper peat quality and water chemistry, rewetting up to the peat surface or only very shallow inundation provides the most suitable conditions for *Sphagnum* recovery (Smolders et al. 2003, Tomassen et al. 2003, Van Duinen et al. 2011). A high water table in the mineral subsoil under bog remnants also has a positive effect on the stability of the water table in the bog remnant, by decreasing the vertical water loss (Schouwenaars 1993, Van der Schaaf 1999). Therefore, the focus of restoration projects in raised bog remnants should be not restricted to retention of rain water to preserve and restore wet and acid conditions typical for bog massifs at micro-scale and meso-scale (Table 1 and Figure 1 in Chapter 1). The restoration strategy should include optimization of the hydrological system up to catchment scale and conservation and restoration of heterogeneity or gradients in environmental conditions typical for raised bog landscapes at macro-scale. This can be achieved by restoring a high groundwater table under bog remnants and in buffer zones adjacent to bog remnants and upward seepage in appropriate sites within and adjacent to bog remnants (Holden et al. 2004, Howie & Tromp-Van Meerveld 2011). Restoration of transitional habitat types may be a realistic target on original locations inside or outside bog remnants, but also at locations adjacent to present-day bog remnants. In addition, perspectives for further development of such gradients are present in fen reserves, where buffered groundwater or surface water and sites in succession to raised bog vegetation are present. Some fen reserves already include hotspots of species typical for bog gradients (Van Kleef et al. 2012).

The variation in vegetation structure and in quality of surface water, interstitial water, and deposited organic matter and peat substrate explain variation in the microinvertebrate and macroinvertebrate species composition (Chapter 2-5). Part of the species spectrum depends on temporary water bodies (Figure 2). The differences in species occurrence between permanent and temporary water bodies are well known and described by many authors (e.g., Galewski 1971, Wiggins et al. 1982). The presence of both temporary and various types of permanent water bodies offers opportunities for many species to survive in an area, e.g. in case of extreme drought (Verberk et al. 2001 and 2002, Moller Pillot 2003). Elevation and stabilisation of the water table by rewetting measures reduces the duration of dry periods of temporary water bodies and thereby the occurrence of invertebrates (Van Duinen et al. 2004).

Comparing variation in species composition and cumulative species richness between restoration sites (1-30 years between rewetting and sampling) and remnant sites (abandoned before 1950) one could expect the number of characteristic species to increase during coming decades. Indeed an increase in the number of rare species and the fauna species quality score (FSQS) was observed in restoration sites (Chapter 2). The average number of species and characteristic species was not significantly different between restoration and remnant sites. Therefore, the larger habitat heterogeneity in remnant sites is considered responsible for the higher cumulative species richness (Verberk et al. 2006, 2010). Habitat conditions and species composition are expected to become more diverse during further development after restoration as this process takes decades to centuries in raised bogs with low dynamics. However, time is not the only factor and it is not sure that succession will finally result in meeting targets (Zedler & Callaway 2000).

Assessment of these developments would be useful for site managers (Zedler & Callaway 2000), requiring monitoring of attributes of the ecosystem and suitable indices and criteria. Assessment of differences and similarities between a reference system and a site under restoration is a first step. We should realize that the current environmental and climate conditions are different from that when raised bogs were developing naturally in the past millennia. Judging the success of

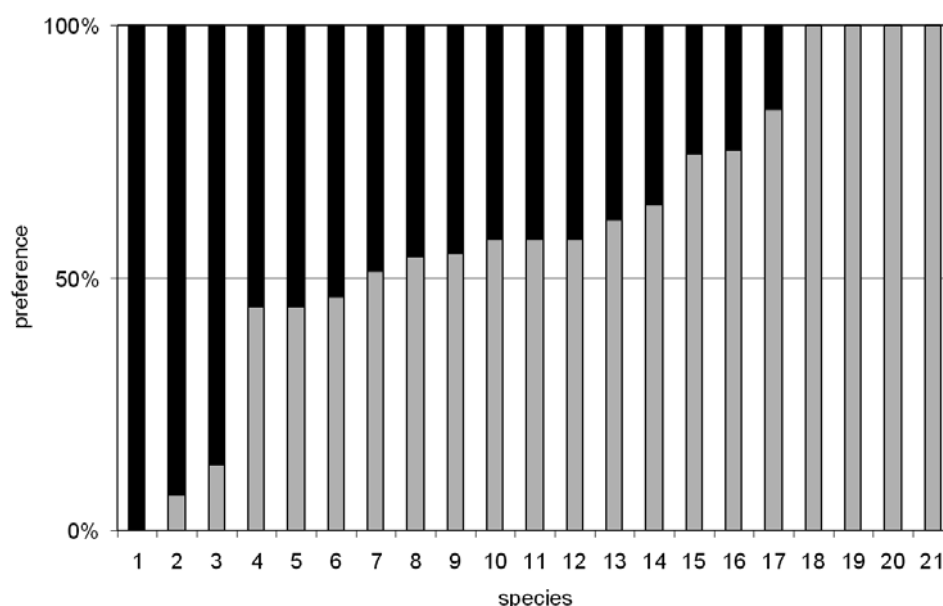


Figure 2. Preference of the 21 species characteristic of bogs and acid water bodies found in the Dutch bog remnant Wierdense Veld for temporary (grey part of the bars) or permanent sites (black part of the bars). The preference was calculated as the frequency of occurrence in permanent and temporary sites, with the sum of these two frequencies converted to 100%. The numbers of sampling sites in which the species were found are given between brackets. 1. *Dytiscus lapponicus* (4); 2. *Hydroporus scalesianus* (7); 3. *Graphoderus zonatus* (11); 4. *Hygrotus decoratus* (22); 5. *Acilius canaliculatus* (12); 6. *Hydroporus umbrosus* (29); 7. *Bidessus spec.* (10); 8. *Enochrus affinis* (18); 9. *Enochrus ochropterus* (11); 10. *Berosus luridus* (4); 11. *Hydroporus obscurus* (8); 12. *Hydroporus melanarius* (4); 13. *Hydroporus tristis* (18); 14. *Agabus melanocornis* (5); 15. *Hydroporus pubescens* (15); 16. *Hydroporus gyllenhalii* (23); 17. *Agabus labiatus* (12); 18. *Rhantus suturellus* (7); 19. *Hydroporus neglectus* (3); 20. *Agabus congener* (3); 21. *Ilybius aenescens* (1). (From: Van Duinen et al. 2004)

peatland restoration must then depend not only on the similarity with a reference system, but on our understanding of functions and processes in raised bog ecosystems (Holden et al. 2004).

Restoration measures that result in preservation of relict populations, preservation or restoration of local groundwater influence, and variation in hydrological regime (permanent and temporary water bodies) will result in a higher invertebrate diversity in bog remnants, including characteristic and rare species. In areas with high atmospheric deposition of N and increased P availability, like in The Netherlands, the rehabilitation of invertebrate communities typical for bog landscapes is limited by excessive nutrient availability and altered food quality (Section 7.3). Here, both hydrological restoration and reduction of the nutrient availability are necessary to rehabilitate typical invertebrate communities.

7.5 Persistence and recolonisation

As natural raised bog development took centuries, patience with respect to the development of the species community may be requested. In the Dutch restoration sites, numbers of rare and characteristic species per site indeed tended to increase with the time elapsed after rewetting

(Chapter 2). Species have to recolonise the restored area from source populations, or persist in the area during the process of degradation and restoration (Van Kleef et al. 2006). Both the mobility (dispersal capacity) and the life history of the species, as well as the preservation or restoration of site conditions -and their temporal and spatial aspects- determine whether species can persist during these processes or can recolonise a rewetted site.

Contrary to most aquatic macroinvertebrates, microinvertebrates, like Rotifera and small crustaceans (Copepoda and Cladocera) are easily spread by wind and animal vectors (Cáceres & Soluk 2002, Cohen & Shurin 2003), often reproduce parthenogenetically, and have a short life cycle that can be completed in one water body (Nogrady et al. 1993, Wetzel 2001). Populations of microinvertebrates, including characteristic species, can indeed persist in raised bog remnants during the process of rewetting or (re-)establish within a relatively short period (less than about 5 years). Environmental variables related to vegetation structure and food quality and availability could explain the dominant pattern in the variation in microinvertebrate assemblages (Chapter 3). This is in contrast to what was found for macroinvertebrates where surface water quality and vegetation composition could explain mainly variation within the groups of restoration sites or remnant sites, but not between these groups of sites (Chapter 2). This indicates that one or more key factors other than surface water quality and vegetation composition determines differences in species composition between remnant and restoration sites. The persistence of characteristic and rare species with poor dispersal capacity during the process of degradation is most likely a key factor.

Man-made bog pools in a Canadian bog restoration project were readily colonised by some aquatic macroinvertebrate species, including bog-associated species (Mazerolle et al. 2006). This concerned aquatic invertebrates with high dispersal abilities, like large aquatic beetles. Less mobile species, like damselflies, chironomids, and caddis flies, that made up an important part of the species composition studied in the Dutch remnant and restoration sites, were not captured in the Canadian study, likely due to the sampling method used (Van Duinen et al. 2007, Mazerolle & Poulin 2007). To avoid that evaluation of the effectiveness of restoration projects end up with only positive or negative conclusions, evaluation should include groups of invertebrates that have different dispersal capacities and life history strategies and may show a differential response.

In regions like in Canada, Sweden, Finland, Estonia, Belarus, and Russia, where natural and near-natural bogs still cover larger areas, chances of recolonisation may be higher than in for instance The Netherlands and parts of Germany and the UK, although further studies are necessary to assess if this is true for more sedentary species (Mazerolle & Poulin 2007, Van Duinen et al. 2007). However, the need for restoration of degraded bog remnants may be higher in more densely populated areas where natural and near-natural bogs are absent. In areas with low chances of recolonisation, persistence of species are a more important aspect in the effectiveness of restoration measures. Vasander et al. (2003) assumed restored habitats to be most likely colonised by typical species if sources of potential colonists are close to restored bog remnants and concluded that sites that are surrounded by target plant and animal species will have priority for restoration. Indeed, the local species pool may to some extent have determined the microinvertebrate assemblages studied in the Dutch bog remnants. Water bodies situated in the same bog remnant were on average more similar to each other than to assemblages from other areas (Chapter 3). Differences between areas may be due to differences in environmental conditions of water bodies, but also to differences in the immigration sequence of species that

is determined by the local species pool. Species already abundant in a bog remnant will be among the first to colonise adjacent, new water bodies created by peat-cutting in the past and more recently by rewetting measures. These ‘early’ species have a high chance to successfully establish a population. Species arriving later may generally have a lower chance to establish a population, as interactions with resident species may prevent them from increasing (priority effects; Jenkins & Buikema 1998, Shurin 2000, Rundle et al. 2002, Cohen & Shurin 2003, Verreydt et al. 2012).

7.6 Species filters acting in bog degradation and restoration

The comparative study presented in this thesis shows that both degradation and restoration have acted as filters (*cf.* Poff 1997) on the aquatic invertebrate community of raised bog landscapes (Figure 3). The loss of natural gradients from the ombrotrophic raised bog massif to the more minerotrophic surroundings caused loss of species characteristic of transitional mires and lags, including several nowadays endangered species (Chapter 4, Van Kleef et al. 2012). In case of small-scale traditional exploitation of bogs, several characteristic species became locally extinct, but a considerable number of characteristic species were able to persist as relict population in the small-scale mosaics of peat cutting pits. In case of industrial exploitation of bogs, hardly any species could live at the drained peat fields.

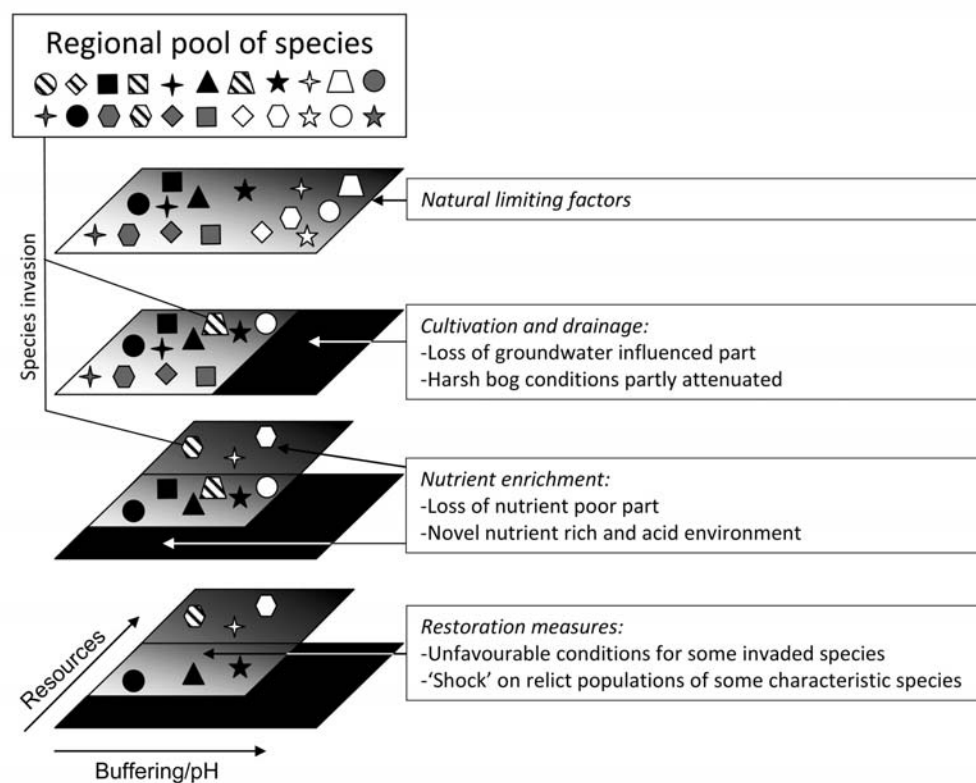


Figure 3. Schematic representation of species filters acting on the aquatic invertebrate community of raised bog landscapes. Different species are represented with different symbols. Short description of the filters in the text boxes at the right side of the figure.

The increased nutrient availability enabled macroinvertebrate species naturally absent from the extremely nutrient limited raised bog massif, or only present in low abundance, to invade bog massifs and to become abundant (Chapter 4 and 5). Several species preferring ombrotrophic water bodies in pristine raised bogs declined or disappeared, either due to changed abiotic conditions or biotic interactions with species that invaded the bog ecosystem. The shifts assessed in species abundance among habitat use groups and especially among congeners show the effect of nutrient availability as filter, enabling some species to increase, while constraining other species.

Rewetting of large-scale cut-over bogs often included inundation after construction of bunds and infilling of drainage ditches. These new water bodies enabled several species, including characteristic and rare species, to establish, depending on the regional and local species pool, dispersal abilities of species, and the habitat conditions (Chapter 3, sections 7.4 and 7.5). However, in small-scale mosaics of old peat cutting pits, where relict populations of characteristic species have been able to persist, restoration measures can also act as a filter, as was found earlier by Van Kleef et al. (2006) and Verberk et al. (2010a). Rewetting enables several species to increase, but may disturb the occurrence of other species (Chapter 2 and 4). Especially bog specialists may be more sensitive to such a disturbance than generalists (Verberk et al. 2010b).

These different filters direct to actions and elements of the bog restoration strategy supporting conservation and restoration of the aquatic invertebrate diversity of raised bog landscapes. The loss of natural gradients between bog massifs and adjacent minerotrophic habitat types and the species dependent on these gradients calls for attention to optimization of the hydrological system up to catchment scale and conservation and restoration of heterogeneity or gradients in environmental conditions typical for raised bog landscapes.

High nutrient availability limits the rehabilitation of aquatic macroinvertebrate communities of raised bog landscapes, like it hampers recovery of characteristic raised bog vegetation (Limpens et al. 2003, Tomassen et al. 2004). Therefore, a further reduction of the atmospheric nitrogen deposition and phosphate concentration in bog remnants is required.

Rewetting may result in a temporal or long-term unsuitable habitat for characteristic, rare and endangered species. Re-colonisation capacity of these species is limited due to habitat fragmentation and limited dispersal ability of species. Therefore, conservation of relict populations of characteristic species currently present in bog remnants, fen reserves and moorland pools is recommended. In restoration projects, preparatory investigations on the functioning of the ecosystem and baseline assessments of species present should be a first step, followed by an appropriate monitoring scheme. Gradual changes over longer periods of time are recommended to avoid shock-effects and to enable species to move within the restoration site, if necessary.

7.7 Answers to main research questions

1. What are the effects of degradation of natural transitions from mineral soil systems to ombrotrophic raised bogs and of nutrient enrichment on aquatic invertebrate communities in raised bog landscapes?

A. The degradation of natural transitions from mineral soil systems to ombrotrophic raised bogs has resulted in the loss of part of the species characteristic of transitional mires and lagg zones, including several species that are nowadays endangered. Groundwater influence

- is lacking in most bog remnants, but is a prerequisite for part of the characteristic species assemblage of raised bog landscapes (Chapter 4 and 5).
- B. Species assemblages characteristic of the most nutrient poor conditions of raised bogs are absent. Nutrient enrichment has enabled part of the species present in pristine bogs to increase their abundance and non-characteristic species to invade acid bog water bodies. In Dutch raised bog remnants, nutrient availability does not limit anymore the occurrence of many invertebrate species absent from pristine ombrotrophic parts of raised bogs, due to the increased nitrogen and phosphorus loads (Chapter 4 and 5).
 - C. Relict populations of many characteristic and rare species have been able to persist the process of degradation in mosaics of peat cutting pits and trenches that remained after 'traditional' exploitation of raised bogs (Chapter 2 and 4).
2. What is the effectiveness of rewetting measures in the rehabilitation of aquatic invertebrate communities in raised bog landscapes?
- A. Aquatic microinvertebrate species, including characteristic raised bog species, which are easily spread by wind and animal vectors and have a relatively short life cycle that can be completed in one water body can either persist in the raised bog remnants during the process of rewetting or (re)establish within a period of less than 5 years (Chapter 3).
 - B. Restoration sites are inhabited by characteristic macroinvertebrate species and rare species, but a considerable number of characteristic and rare species are only found at remnant sites which have not been affected by industrial exploitation nor large-scale rewetting. These remnant sites include considerably more variation in aquatic macroinvertebrate species assemblages (Chapter 2).
 - C. Numbers of rare and characteristic species per site tend to increase with the time elapsed after rewetting. However, restoration measures will not automatically result in restoration of a more or less complete macroinvertebrate species spectrum (Chapter 2 and 4).
3. Which environmental factors facilitate or limit the rehabilitation of aquatic invertebrate communities in raised bog landscapes?
- A. Increased nutrient availability (N and P) in Dutch raised bog remnants has resulted in the loss of the macroinvertebrate assemblages characteristic of nutrient-poor ombrotrophic raised bog pools. The aquatic macroinvertebrate abundance is increased in Dutch bog remnants, primarily due to species that are absent from pristine bogs and species that in unpolluted bog landscapes prefer transitional mires which have a higher nutrient availability (Chapter 4).
 - B. Cultivation and drainage have caused a decrease or loss of groundwater influence in most raised bog remnants. Several macroinvertebrate species that in pristine bog landscapes only occur in transitional mires and lags are absent or rare in Dutch bog remnants and endangered in The Netherlands and neighbouring countries (Chapter 4 and 5).
 - C. Rewetting may result in temporal or long-term unsuitable habitat for characteristic and rare species. The (re)colonisation by these species is limited by the distance to nearest source populations (habitat fragmentation) and poor dispersal abilities of species (Chapter 2 and 3).

7.8 Recommendations for restoration practice

1. High nutrient availability limits the rehabilitation of aquatic invertebrate communities of raised bog landscapes. Therefore, a further reduction of atmospheric N-deposition and the P load in bog remnants is required (Chapter 4, 5 and section 7.3).

2. Intensive drainage or groundwater extraction limit the rehabilitation of aquatic invertebrate communities, including endangered species, characteristic of groundwater influenced parts of raised bog landscapes (Chapter 4 and 5). Therefore, restoration of larger hydrological systems and transitional habitat types is required. Restoration of gradients in groundwater influence could be carried out at original locations inside or outside bog remnants, but also in buffer zones adjacent to present-day bog remnants. Construction of buffer zones is a measure that improves hydrological conditions inside bog remnants and may also create gradients in acid buffering and nutrient availability. Bog remnants must be protected against inlet of surface water enriched in sulphur or nutrients. Suitable sites for further development of gradients may be found in fen reserves, where buffered groundwater or surface water and sites in succession to raised bog vegetation are present. Some fen reserves already include hotspots of species typical for bog gradients.
3. Rewetting may result in temporal or long-term unsuitable habitat for characteristic and rare species with poor (re)colonisation capacity. Therefore, the conservation of relict populations of characteristic species present in bog remnants, fen reserves and moorland pools is recommended to be included in restoration strategies. In restoration projects, preparatory investigations on the functioning of the ecosystem and assessments of species present should be a first step, followed by an appropriate monitoring scheme. Gradual changes over longer periods of time are recommended to avoid shock-effects and to enable species to move within the restoration site, if necessary.

Recommendations for restoration practice resulting from the present study as well as hydrological, chemical, and vegetation studies are described in more detail by Van Duinen et al. (2011).

7.9 Recommendations for further research

Further research is recommended focussing on mechanisms that cause the species responses observed in this study. These include:

1. Interspecific competition -especially among congeners- as affected by increased nutrient availability and reduced buffering capacity.
2. The role of changes in quality of plant material (stoichiometry of macro- and micro-nutrients) in species responses and to further reveal the role of invertebrates in the carbon cycle and the importance of methane in the invertebrate food web in pristine and eutrophied raised bogs.
3. How the invertebrate species composition is affected by priority effects and overlap in species' niches.

To further improve restoration practice, an evaluation of the effects of implementation of the recommendations for management practice given in this thesis is necessary. Does the (re)construction of gradients and gradual or small scaled changes result in expected species responses? To give more specific guidelines on the restoration and management of habitat heterogeneity, study on the use of different parts of the bog landscape by fauna is necessary.

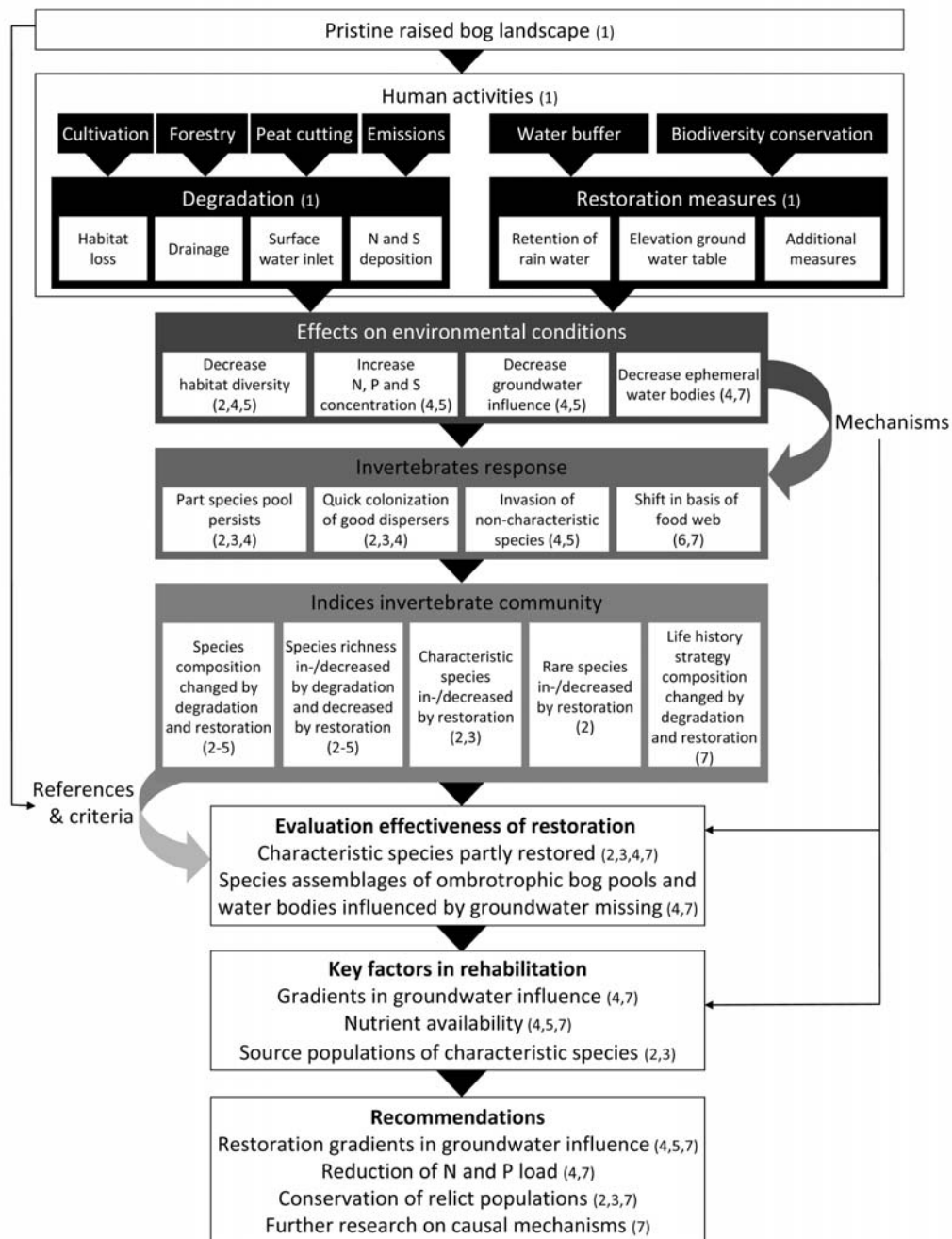


Figure 4. Schematic representation of the past and current human activities that impact the invertebrate community of raised bog landscapes. Changes in environmental conditions, the invertebrates' responses on these changes and the corresponding changes in indices of the invertebrate community assessed in this thesis are summarized in the central part of the scheme. The numbers of the chapters in which the respective aspects are described are given between brackets. The lower part of the scheme presents the conclusions and recommendations for restoration management and further research.

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Summary

◀ The larvae of the bog hawker *Aeshna subarctica* develop in floating peat mosses. Here a female taking a rest on a pine tree in the largest swamp system of the northern hemisphere, the Great Vasyugan Mire, Western Siberia.

Summary

Peatlands are considered as one of the most important natural ecosystems of the world, because of their characteristic biodiversity and highly valued ecosystem services. However, the degradation of peatlands is ongoing due to cultivation, drainage, afforestation, peat extraction, increased atmospheric deposition of nitrogen and sulphur, and climate change. Restoration measures are carried out in raised bog remnants abandoned after peat extraction or forestry. These measures include rainwater retention, aiming at regenerating a self-sustaining bog ecosystem or maintaining viable populations of characteristic species.

An introduction to characteristics of raised bog landscapes and the causes of degradation is presented in **Chapter 1**. An essential feature of active raised bogs is the accumulation and storage of peat. Hydrology and *Sphagnum* mosses play a key role in this feature. The composition of bog landscapes at different spatial scales is important for faunal diversity characteristic of raised bogs. Complete bog landscapes are characterised by both an ombrotrophic bog massif that is acid and extremely nutrient limited, as well as gradients from the bog massif to the more minerotrophic surroundings, where these harsh conditions are gradually attenuated. The presence of these gradients particularly contribute to a high faunal diversity of the bog landscape.

Many investigations on raised bog degradation and restoration focus on bog vegetation and key factors involved in its recovery. The impact of both degradation and restoration measures on invertebrate fauna remained largely unknown. The aim of this thesis is to assess the effectiveness of restoration measures and the key factors involved in the degradation and rehabilitation of aquatic invertebrate communities in raised bog landscapes in order to improve restoration management practices.

To assess whether raised bog restoration measures contribute to the conservation and restoration of the fauna diversity, aquatic macroinvertebrate species assemblages were studied in Dutch raised bog remnants. The fauna diversity was compared between A) water bodies created by rewetting measures and B) water bodies which have not been subject to restoration measures, but are remnants of peat cutting pits and trenches that remained after ‘traditional’ exploitation of raised bogs (**Chapter 2**). The restoration sites are inhabited by characteristic raised bog species and rare species, but their numbers are higher at the remnant sites not affected by restoration management. Forty percent of the characteristic and rare fauna species are only found at the remnant sites. The remnant sites show much more variation in macroinvertebrate species assemblages and a higher cumulative species richness. The number of characteristic macroinvertebrate species is not clearly related to the presence of a characteristic raised bog vegetation. In restoration sites, the numbers of rare and characteristic species per site tend to increase with the time elapsed after rewetting, indicating a colonisation process. However, restoration measures will not automatically result in recovery of a more or less complete macroinvertebrate species spectrum. The restoration measures have so far resulted in habitats for only two third of the characteristic species.

Different species or species groups may respond differently to restoration measures. Contrary to most aquatic macroinvertebrates, microinvertebrates, such as Rotifera and small crustaceans (Copepoda and Cladocera), are easily spread by wind and animal vectors and have a short life cycle that can be completed in one water body. **Chapter 3** evaluates effects of restoration measures in raised bog remnants on the species composition of Rotifera and microcrustaceans. The species assemblages, total numbers of species and numbers of characteristic raised bog species do not differ between the restoration and remnant sites. Environmental variables related to vegetation

structure and food quality and availability can explain the dominant pattern in the variation in microinvertebrate assemblages. Furthermore, the species assemblages of water bodies situated in the same area are on average more similar to each other than to assemblages from other areas. These differences between areas may be due to differences in environmental conditions of water bodies and local differences in the sequence and timing of colonisation by which niches were already occupied (priority effects). Species already abundant in a bog remnant may be among the first to colonise new water bodies and to successfully establish a population. Species arriving later may generally have a lower chance to establish a population, as interactions with resident species may prevent them from increasing numbers. In contrast to findings on aquatic macroinvertebrates, populations of microinvertebrate species, including characteristic species, can either persist in the raised bog remnants during the process of rewetting or (re)establish within a period of time less than five years.

Raised bog landscapes are degraded by reclamation, causing loss of natural gradients between ombrotrophic bog massifs and the surrounding minerotrophic landscape. In addition, bogs are vulnerable to nutrient enrichment. To restore degraded bogs rainwater is retained, aiming at recovery of a *Sphagnum*-dominated vegetation. Two complementary hypotheses are tested by comparison of aquatic macroinvertebrate assemblages between pristine reference systems in Estonia and degraded bog remnants in The Netherlands (**Chapter 4**). The latter have a 4-6 fold increase of N and P compounds in surface water. The first hypothesis is that increased nutrient availability enables invertebrate species naturally absent from extremely nutrient limited bog massifs to become abundant in nutrient enriched bog remnants. The second hypothesis is that invertebrate assemblages characteristic of gradients from ombrotrophic bogs to minerotrophic surroundings hardly profit from the restoration practice focusing on ombrotrophic conditions solely. The aquatic macroinvertebrate abundance is higher in bog remnants in The Netherlands than in ombrotrophic water bodies and in transitional mires in Estonia. This difference in abundance is indeed primarily due to species that are absent from Estonian bogs and species preferring transitional mires. In water bodies created by rewetting measures the abundance of species preferring nutrient poor ombrotrophic pools is increased compared to water bodies remaining after historical use of bogs, like peat cutting pits. However, the cumulative species richness of species preferring nutrient poor ombrotrophic pools is not increased. The cumulative species richness and abundance of species preferring more minerotrophic parts of bog gradients are decreased compared to the remnant sites. In contrast to restoration sites, the group of remnant sites includes the whole gradient from acid water bodies in bog massifs to the lagg of complete raised bog landscapes.

Further insight in how loss of groundwater influence and increase in nutrient availability affect the species occurrence has been obtained by comparing studying aquatic oligochaete species composition within and between pristine raised bogs in Estonia and raised bog remnants in The Netherlands (**Chapter 5**). Within the pristine bog landscape a distinct pattern in the species assemblage is present. In the most nutrient-poor water bodies in the ombrotrophic raised bog, only one oligochaete species was present. This is an acid-tolerant species that almost never matures and reproduces by fragmentation. In Estonia, other oligochaete species are limited to more minerotrophic water bodies, which have a higher decomposition rate of dead organic matter and, consequently, higher nutrient availability. Comparison of the occurrence of three oligochaete species between Estonian and Dutch sites indicate that these three species respond differently to the increased nutrient availability in The Netherlands, which can be linked to differences in their diets. In The Netherlands, the occurrence of oligochaetes appeared to be not

Summary

limited anymore by nutrient availability. The lagg zone is the most species-rich part of a pristine bog landscape. Most of these lagg zone species are not present in Dutch bog remnants as this part of the bog landscape has long been cultivated. Overall, it is concluded that the degradation of Dutch raised bogs has resulted in the loss of both the nutrient-poor parts of the landscape and the special lagg conditions, clearly reflected by oligochaete occurrence.

Raised bog pools are naturally extremely nutrient poor and rich in humic substances, limiting primary production. To assess the base of the invertebrate food web in bog pools stable isotopic values of primary producers, dead organic matter, and invertebrates, as well as the composition and stable carbon isotope ratio of phospholipid fatty acids (PLFAs) were measured in pristine bog pools in Estonia (**Chapter 6**). The stable isotopic values show the presence of multiple trophic levels and a differential use of basal food sources by the invertebrates, both between different species and within species, among different individuals and size classes. Carnivorous and omnivorous invertebrates assimilate polyunsaturated fatty acids (PUFAs) derived from algae, and possibly macrophytes, as well as fatty acids that are specific for methane oxidizing bacteria (MOB). A considerable part of the bacterial biomass conveyed to higher trophic levels in the bog pools likely originates from MOB. Protozoa and zooplankton synthesizing PUFAs commonly used as biomarkers for algae may play a role in this pathway. Pelagic zooplankton seems to rely more on bacteria, whereas for insects algae (periphyton and possibly phytoplankton) are more important. The relatively depleted $\delta^{13}\text{C}$ values of PUFAs in invertebrates point to the use of algae that possibly derived carbon from MOB. Therefore, depleted $\delta^{13}\text{C}$ values of invertebrates do not necessarily implicate a direct pathway between MOB and these invertebrates, but algal food sources forming an intermediate level. Stable isotopic values measured in potential basal carbon sources and aquatic invertebrates in Dutch bogs indicate that increasing nutrient availability results in shifts in the use of basal carbon sources. Other types of algae and dead organic matter may support an increasing proportion of the invertebrate community, whereas the role of methane and methanotrophic bacteria at the basis of the food web may decrease.

The effectiveness of restoration measures and the key factors involved in the degradation and rehabilitation of aquatic invertebrate communities in raised bog landscapes are discussed in the synthesis (**Chapter 7**). This chapter concludes with recommendations for restoration management and further research:

- Measures to further reduce atmospheric nitrogen deposition and to reduce the elevated phosphate concentration in Dutch bog remnants are required to allow for further rehabilitation of aquatic macroinvertebrate communities of raised bog landscapes.
- Restoration of gradients from groundwater influenced to acid parts of raised bog landscapes. Opportunities may be found in buffer zones on former agricultural grounds adjacent to current bog remnants, as well as in fen reserves that include patches in succession to bog.
- Conservation of relict populations of characteristic species of raised bog landscapes currently present in bog remnants, as well as fen reserves and moorland pools.
- Further research focussing on mechanisms that cause the species responses observed in this study, as well as an evaluation of the effects of implementation of the recommendations for the management practice given in this thesis.



Samenvatting

◀ Construction of a new bund (or dyke) of sand, covered with peat, to preserve the remaining peat massif in the bog remnant Engbertsdijksvenen, The Netherlands.

Samenvatting

Veengebieden worden beschouwd als een van de belangrijkste natuurlijke ecosystemen van de aarde wegens hun kenmerkende biodiversiteit en hoog gewaardeerde ecosysteemdiensten. Een essentieel kenmerk van levende hoogvenen is de vorming en opslag van veen. Hydrologische processen en veenmossen spelen hierbij een cruciale rol. De aantasting van venen gaat echter door als gevolg van ontginning, ontwatering, bosbouw, veenwinning, verhoogde atmosferische depositie van stikstof en zwavel en klimaatverandering. Herstelmaatregelen worden uitgevoerd in hoogveenrestanten nadat veenwinning of bosbouw is beëindigd. Deze maatregelen zijn gericht op het vasthouden van regenwater, met als doel het regenereren van een zichzelf in stand houdend hoogveenecosysteem en de instandhouding van levensvatbare populaties van kenmerkende soorten.

Kenmerken van intacte hoogveenlandschappen en hoe aantastingen daarop ingrijpen worden besproken in **hoofdstuk 1**. Complete hoogveenlandschappen worden gekenmerkt door zowel een uitsluitend door regenwater gevoede (ombrotrofe) hoogveenkern, die zuur is en waar de beschikbaarheid van voedingsstoffen uiterst beperkt is, als overgangen (gradiënten) vanuit de hoogveenkern naar de mineraalrijkere omgeving, waarin de beperkende omstandigheden geleidelijk afnemen. De aanwezigheid van de hele gradiënt van de hoogveenkern tot en met de rand (lagg-zone) is essentieel voor een grote faunadiversiteit van het hoogveenlandschap.

Veel onderzoek naar de aantasting en het herstel van hoogvenen is gericht op de hoogveenvegetatie en factoren die sturend zijn in het herstel daarvan. De effecten van zowel aantastingen, als herstelmaatregelen op de ongewervelde fauna zijn grotendeels onbekend. Het doel van dit proefschrift is het vaststellen van de effectiviteit van herstelmaatregelen voor ongewervelde waterdieren (watermacrofauna), zoals libellenlarven, waterwantsen, waterkevers en larven van dansmuggen. Om het herstelbeheer van hoogveenrestanten te kunnen verbeteren, is kennis verzameld over de belangrijkste factoren in de aantasting en het herstel van deze diergemeenschappen.

Om vast te stellen of herstelmaatregelen in hoogveenrestanten bijdragen aan het behoud en herstel van de faunadiversiteit is de soortensamenstelling van de watermacrofauna onderzocht in intacte hoogveenlandschappen in Estland en hoogveenrestanten in Nederland. In **hoofdstuk 2** wordt de soortensamenstelling van de watermacrofauna vergeleken tussen A) herstelwateren, die door vernattingsmaatregelen zijn gevormd, en B) relictwateren in Nederlandse hoogveenrestanten die niet zijn onderworpen aan herstelmaatregelen. Deze relictwateren zijn overblijfselen van veenputten en greppels die zijn gegraven ten tijde van het vroegere ('traditionele') gebruik van hoogveen. De herstelwateren worden bewoond door karakteristieke hoogveensoorten en zeldzame soorten, maar het aantal soorten is hoger in de relictwateren. Veertig procent van de karakteristieke en zeldzame diersoorten werden alleen aangetroffen in deze relictwateren. De relictwateren tonen veel meer variatie in de samenstelling van de watermacrofauna en een hogere cumulatieve soortenrijkdom. Het aantal karakteristieke macrofaunasoorten is niet duidelijk gerelateerd aan de aanwezigheid van een karakteristieke hoogveenvegetatie. In de herstelwateren lijkt het aantal zeldzame en karakteristieke soorten per locatie toe te nemen met de tijd na vernatting. De herstelmaatregelen hebben tot nu toe geresulteerd in leefgebieden voor twee derde van de karakteristieke macrofaunasoorten. Dit wijst op het optreden van een relatief langzaam proces van kolonisatie en geeft aan dat de relictwateren belangrijk zijn voor de overleving van een deel van de karakteristieke en zeldzame soorten. Herstelmaatregelen zullen niet automatisch leiden tot herstel van een min of meer volledig soortenspectrum van de

watermacrofauna van hoogveenlandschappen, omdat een deel van de soorten afhankelijk is van gradiënten in het hoogveenlandschap.

De mate waarin soorten nieuwe leefgebieden kunnen koloniseren en de eisen die ze daaraan stellen verschillen per soortgroep. Daardoor kunnen ze verschillend reageren op herstelmaatregelen. In tegenstelling tot de meeste watermacrofaunasoorten worden kleine ongewervelde waterdieren, zoals raderdieren (Rotifera), eenoogkreeftjes (Copepoda) en watervlooien (Cladocera), gemakkelijk verspreid door de wind of grotere dieren en hebben zij een korte levenscyclus die voltooid kan worden binnen één water. **Hoofdstuk 3** evalueert effecten van herstelmaatregelen in Nederlandse hoogveenrestanten op de soortensamenstelling van raderdieren, eenoogkreeftjes en watervlooien. De soortensamenstelling van deze microfauna, het totaal aantal soorten en het aantal karakteristieke soorten verschilde niet tussen de herstel- en relictwateren. Omgevingsvariabelen gerelateerd aan vegetatiestructuur en kwaliteit en beschikbaarheid van voedsel voor deze microfaunagroepen kunnen het dominante patroon in de variatie in de microfaunasamenstelling verklaren. De soortensamenstelling in wateren in hetzelfde gebied lijken gemiddeld meer op elkaar dan op de soortensamenstelling in andere gebieden. Deze verschillen tussen gebieden kunnen veroorzaakt zijn door verschillen in milieuomstandigheden van de wateren en lokale verschillen in de volgorde van kolonisatie door soorten. Daarbij kunnen soorten die in een hoogveenrestant talrijk zijn als eerste nieuwe of ingrijpend veranderde wateren koloniseren en met succes een populatie vestigen. Soorten die zulke wateren pas later bereiken, hebben over het algemeen een kleinere kans zich te vestigen, doordat interacties met gevestigde soorten hun populatietoename belemmeren (prioriteitseffect). In tegenstelling tot de watermacrofauna kunnen populaties van microfaunasoorten, waaronder kenmerkende soorten, zich in de hoogveenrestanten handhaven gedurende het proces van vernatting of zich herstellen binnen een periode van minder dan vijf jaar.

Door ontginning zijn natuurlijke gradiënten tussen ombrotrofe hoogveenkernen en het omringende mineraalrijkere landschap verloren gegaan. Bovendien zijn de van nature uiterst voedselarme hoogvenen kwetsbaar voor verrijking met voedingsstoffen (nutriënten) door verhoogde atmosferische depositie en verhoogde afbraak van het veenpakket door ontwatering. Om gedegradeerde hoogvenen te herstellen wordt regenwater vastgehouden, met als doel het herstel van een door veenmossen gedomineerde vegetatie. Twee complementaire hypothesen worden getoetst door vergelijking van de watermacrofauna tussen intacte referentiesystemen in Estland en aangetaste hoogveenrestanten in Nederland (**hoofdstuk 4**). In deze hoogveenrestanten is sprake van een vier- tot zesvoudige toename van de concentratie van stikstof- en fosforverbindingen in het oppervlaktewater in vergelijking met de Estlandse referentie. De eerste hypothese is dat de toegenomen beschikbaarheid van deze voedingsstoffen heeft geleid tot een facilitatie van macrofaunasoorten die van nature ontbreken in de uiterst voedselarme hoogveenwateren. De tweede hypothese is dat herstelmaatregelen nauwelijks hebben geleid tot een verbetering voor macrofaunasoorten die karakteristiek zijn voor intacte gradiënten tussen ombrotrofe hoogveenkernen en de minerotrofe omgeving, omdat deze maatregelen vaak uitsluitend gericht zijn op herstel van ombrotrofe omstandigheden. Uit de vergelijking blijkt dat de watermacrofauna talrijker is in Nederlandse hoogveenrestanten dan in intacte ombrotrofe hoogveenwateren en overgangsvennen in Estland. Dit verschil in dichtheid (abundantie) is inderdaad in de eerste plaats te wijten aan soorten die ontbreken in Estlandse hoogveenlandschappen en soorten met een voorkeur voor een hogere beschikbaarheid van mineralen en voedingsstoffen. In herstelwateren is de dichtheid van soorten met een voorkeur

Samenvatting

voor voedselarme ombrotrofe wateren toegenomen ten opzichte van relictwateren. Dat geldt echter niet voor de soortenrijkdom. Voor de soorten met een voorkeur voor meer minerotrofe delen van hoogveengradiënten zijn het totaal aantal soorten en de dichtheden juist lager in herstelwateren dan in relictwateren. In tegenstelling tot herstelwateren omvat de groep van relictwateren de hele gradiënt van zure wateren in hoogveenkernen tot en met de rand van complete hoogveenlandschappen.

Meer inzicht in hoe het verlies van de grondwaterinvloed en verhoging van de beschikbaarheid van voedingsstoffen het voorkomen van soorten beïnvloeden, is verkregen door het vergelijken van de soortensamenstelling van borstelwormen (Oligochaeta) binnen en tussen intacte hoogveenlandschappen in Estland en hoogveenrestanten in Nederland (**hoofdstuk 5**). Binnen het intacte hoogveenlandschap bestaat een duidelijk patroon in de soortensamenstelling. In de meest voedselarme wateren in de ombrotrofe hoogveenkern is slechts één soort borstelworm (*Cognettia sphagnetorum*) aangetroffen. Dit is een zuurtolerante soort die bijna nooit volwassen wordt en zich ongeslachtelijk voortplant door fragmentatie. In Estland zijn andere soorten borstelwormen beperkt tot de meer minerotrofe wateren, waar de afbraaksnelheid van dood organisch materiaal hoger is en daardoor de beschikbaarheid van voedingsstoffen hoger is. Vergelijking van het voorkomen van drie soorten borstelwormen tussen Estlandse en Nederlandse hoogveenwateren toont aan dat deze drie soorten verschillend reageren op de verhoogde beschikbaarheid van voedingsstoffen in Nederland, hetgeen gerelateerd kan worden aan verschillen in hun dieet. In Nederland blijkt het voorkomen van borstelwormen niet meer beperkt te worden door de beschikbaarheid van voedingsstoffen. De lagg-zone is het meest soortenrijke deel van een intact hoogveenlandschap. De meeste van deze lagg-zonesoorten zijn niet aanwezig in de Nederlandse hoogveenrestanten, doordat dit deel van het hoogveenlandschap sinds lang in cultuur is gebracht. De degradatie van de Nederlandse hoogvenen heeft geresulteerd in het verlies van zowel de voedselarme delen van het landschap, als de bijzondere omstandigheden van de randen van hoogvenen (lagg-zones), wat ook duidelijk tot uiting komt in het voorkomen van soorten borstelwormen.

Hoogveenwateren zijn van nature zeer voedselarm en donker gekleurd door humuszuren en humusdeeltjes, waardoor de primaire productie door planten wordt beperkt. Om de basis van het voedselweb van ongewervelde dieren in hoogveenwateren vast te stellen, zijn de verhoudingen tussen stabiele isotopen van koolstof en stikstof bepaald van primaire producenten, dood organisch materiaal en ongewervelden uit drie poelen in een intacte hoogveenkern in Estland. Daarnaast is van een aantal soorten ongewervelden de samenstelling van fosfolipide vetzuren (PLFAs) en de verhouding tussen stabiele koolstofisotopen van deze PLFAs bepaald (**hoofdstuk 6**). De stabiele-isotopenratio's tonen de aanwezigheid van meerdere trofische niveaus in deze hoogveenpoelen aan en een verschillend gebruik van basale voedselbronnen door de ongewervelde dieren, zowel tussen de verschillende soorten, als tussen verschillende individuen en grootteklassen binnen soorten. Carnivore en omnivore ongewervelden verwerken zowel meervoudig onverzadigde vetzuren (PUFAs) uit algen en mogelijk macrofyten, als vetzuren die specifiek zijn voor methaanoxiderende bacteriën (MOB). Een aanzienlijk deel van de bacteriële biomassa die terecht komt in hogere trofische niveaus is waarschijnlijk afkomstig van deze MOB. Protozoën en zoöplankton, die PUFAs synthetiseren die gebruikt worden als biomarkers voor algen, spelen mogelijk een rol in deze route. Pelagisch zoöplankton lijkt zich meer te voeden met bacteriën, terwijl voor insecten algen belangrijker zijn (perifyton en mogelijk fytoplankton). De relatief zeer negatieve $\delta^{13}\text{C}$ waarden van PUFAs in ongewervelden wijzen op het gebruik van algen die mogelijk koolstof verkrijgen van MOB. Daarom impliceren zulke negatieve $\delta^{13}\text{C}$

waarden van ongewervelde dieren niet per se een directe weg tussen MOB en deze ongewervelde dieren, maar kunnen algen als voedselbron dienen en een tussenliggend trofisch niveau vormen. Uit stabiele-isotopenratio's van mogelijke basale koolstofbronnen en ongewervelde dieren in Nederlandse hoogveenwateren blijkt dat toenemende beschikbaarheid van voedingsstoffen resulteert in verschuivingen in het gebruik van basale koolstofbronnen. Andere soorten algen en dood organisch materiaal kunnen bij toename van de beschikbaarheid van voedingsstoffen mogelijk een steeds groter deel van de gemeenschap van ongewervelden voeden, terwijl de rol van methaan en MOB in de basis van het voedselweb juist kan afnemen.

De effectiviteit van herstelmaatregelen en de belangrijkste factoren die betrokken zijn bij de aantasting en het herstel van gemeenschappen van ongewervelde waterdieren in hoogveenlandschappen worden besproken in de synthese (**hoofdstuk 7**). Dit hoofdstuk wordt afgesloten met aanbevelingen voor het herstelbeheer en verder onderzoek:

- Maatregelen om de atmosferische stikstofdepositie verder te verminderen en de verhoogde fosfaatconcentratie te verminderen in de Nederlands hoogveenrestanten zijn noodzakelijk voor verder herstel van watermacrofaunagemeenschappen van hoogveenlandschappen.
- Herstel van gradiënten tussen zure en door gebufferd grondwater beïnvloede delen van hoogveenlandschappen. Mogelijkheden kunnen worden gevonden in bufferzones op voormalige landbouwgronden grenzend aan de huidige hoogveenrestanten, evenals in laagvenen waarin delen met successie naar hoogveen aanwezig zijn.
- Behoud van relictpopulaties van kenmerkende soorten van hoogveenlandschappen die momenteel aanwezig zijn in hoogveenrestanten, laagvenen en vennen.
- Verder onderzoek gericht op het ophelderen van mechanismen die ten grondslag liggen aan de respons van soorten op aantasting en herstelmaatregelen zoals die in dit onderzoek zijn vastgesteld, alsook een evaluatie van de effecten van de uitvoering van de aanbevelingen voor de beheerspraktijk die in dit proefschrift zijn gegeven.



Kokkuvõte

◀ Diversity of nanotopes (or mire micro-forms) in the centre of the raised bog Männikjärve, Estonia.

Kokkuvõte

Eripärane elurikkus ja väärtuslikud ökosüsteemsed hüved teevad soodest ühed maailma olulisemad ökosüsteemid. Samas jätkub soode degradeerumine, mida põhjustab põllumajandus, metsandus, kuivendamine, turba kaevandamine, õhusaastest tulenev lämmastiku ja väe saastekoormuse suurenemine ja kliima muutused. Pärast turbakaevandamise lõpetamist taastatakse jääksood reeglina märgalaks või kasutatakse neid metsa kasvatamiseks. Rabakoosluste taastamiseks kasutatakse toitainetevaeste sadevete kogumist jääksöö turbaalale eesmärgiga taastada isetoimiv ökosüsteem koos elupaigale tüüpiliste liikide elujõuliste asurkondadega.

1. osas antakse sissejuhatav ülevaade rabadest ning nende hävimise põhjustest. Looduslike rabade iseloomulikuks omaduseks on turba kasv ja säilimine, mille võtmeteguriteks on veerežiim ja turbasamblad (*Sphagnum*). Rabamaastiku erinevas mastaabis muistrid määravad sealse fauna mitmekesisuse. Terviklikku rabamaastikku kuuluvad nii toitainetevaesed sademetoitelised rabamassiivid kui ka rohkematoitelised rabamassiive ümbritsevad siirdesood, mis leevendavad raba karme tingimusi. Sellised toitainete kättesaadavuse muutusastmed (gradiendid) põhjustavad koos rabamaastiku fauna mitmekesisuse.

Enamik rabade degradeerumist ja taastamist käsitlevaid uuringuid keskendub taimkattele ja selle taastamise võtmeteguritele. Nii degradeerumise kui taastamise mõju raba selgrootute faunale on uuritud väga vähe. Käesolevate teeside eesmärgiks on hinnata taastamistegevuste tulemuslikust ja mõjuteguridest, mis kujundavad nii degradeerunud kui taastatud rabade selgrootute kooslusi, eesmärgiga tõhustada raba taastamise meetodeid.

Hindamaks, kas rabade taastamismeetmed mõjutavad selgrootute fauna mitmekesisust, uuriti suurselgrootute kooslusi Hollandi jäänukrabades. Fauna mitmekesisust võrreldi sooveekogudes, mis tekkisid taastamisala üleujutades (A) ja traditsioonilise turbakaevandamise käigus tekkinud jäänukveekogudes (B), mis ei ole olnud taastamise objektiks (**2. osas**). Taastamisel tekkinud veekogudes esinesid nii rabale tüüpilised kui ka haruldased liigid, kuid nende arvukus oli jäänukrabade väikeveekogudes kõrgem. Nelikümmend protsenti rabale tüüpilistest ja haruldastest liikidest leiti anult jäänukrabade veekogudest. Jäänukrabade veekogude suurselgrootute koosluste varieeruvus ja kumulatiivne liigirikkus olid suuremad. Tüüpiliste suurselgrootute liikide arv ei olnud seotud rabale tüüpilise taimkatte esinemisega. Taastamisaladel sõltus haruldaste ja tüüpiliste liikide esinemine üleujutamisest möödunud ajast, tulenedes asustamisprotsessist. Samas ei taganud taastamismeetmed kogu tüüpilise selgrootute fauna taastumist – taastatud aladel leiti vaid 2/3 tüüpiliste liikide koguarvust.

Taastamine mõjutas erinevaid liike ja liigigruppe erinevalt. Võrreldes enamike suurselgrootutega levivad väikeselgrootud nagu keriloomad (*Rotifera*), aerjalgsed (*Copepoda*) ja vesikirbulised (*Cladocera*) tuule ja loomade abil kergemini ning on samas veekogus võimelised elutsükli läbima kiiremini. **3. osas** käsitletakse taastamise mõju keriloomadele, aerjalgsetele ja vesikirbuliste kooslustele. Koosluse liigiline koosseis, liikide koguarv ja tüüpiliste liikide esinemine taastamisalade ja jäänukrabade veekogudes ei erinenud. Väikeselgrootute koosluste varieerumist selgitasid põhiliselt keskkonnatunnused, taimkatte struktuur ja toidu kvaliteet ning kättesaadavus. Samuti olid samas piirkonnas erinevate väikeveekogude väikeselgrootute kooslused sarnasemad kui erinevates piirkondades. Erinevused piirkondade vahel on põhjustatud erinevatest keskkonnatingimustest veekogudes ja lokaalsetest asustamisprotsessidest. Jäänuksoo veekogude arvukad liigid on ka esimesteks taastatud veekogu asustavateks liikideks, kes moodustavad seal püsivaid asurkondi. Hiljem uude elupaika saabunud liikidel on juba tekkinud

konkurentsi tõttu raskem moodustada püsivat asurkonda. Erinevalt suurselgrootutest taastasid väikeselgrootute kooslused vähem kui viie aasta jooksul peale veereziimi taastamist.

Degradeerunud rabamaastikes puuduvad looduslikud muutusastmed ombotroofsete rabamassiivide ja ümbritseva minerotroofse maastiku vahel ning säilinud looduslikud vähetoitelised rabad on mõjutatud toitainete kuhjumisest. Degradeerunud raba taastamiseks püütakse koguda turbamaale sadevett eesmärgiga taastada turbasammalde kasv. **4. osas** kontrollitakse kaht üksteist täiendavat hüpoteesi võrreldes suurselgrootute kooslusi Eesti looduslikes rabades ning Hollandi degradeerunud jäänukrabades. Viimastes on pinnavete lämmastiku ja fosfori ühendite sisaldus 4-6 korda suurem. Esimese hüpoteesi järgi võimaldab suurenenud toitainete kättesaadavus selgrootute liikidel, kes puuduvad toitainete poolest erakordselt vaestes looduslikes rabades, asustada arvukalt toitainete poolest rikkaid jäänuksooid. Teise hüpoteesi järgi selgrootutute kooslused, kes on tüüpilised loodusliku sookompleksi erinevate toitumistingimustega muutusastmetele, ei taastu kui lähtutakse ainult vähetoitelise rabakoosluse sihtaastamise eesmärgist arvestamata looduslikule kompleksile iseloomulikke muutusastmeid. Veeselgrootute arvukus oli Hollandi jäänukrabades suurem kui Eesti vähetoitelistes rabalaugastes ja siirdesoodes. Erinevus tulenes liikidest, kes Eesti rabades puuduvad ning siirdesoid eelistavatest liikidest. Raba taastamisel loodud veekogudes suurenes vähetoitelistele rabalaugastele iseloomulike liikide osakaal võrreldes traditsioonilise turbakaevandamise tulemusel tekkinud jäänukveekogudega. Siiski vähetoitelistele rabalaugastele iseloomulike liikide kuhjuv liigirikkus ei suurenenud ning minerotroofsetele soolupaikadele iseloomulike liikide kuhjuv liigirikkus oli väiksem kui jäänukrabade veekogudes. Taastatud aladega võrreldes esines jäänuksoodes erineva toitumistingimustega muutusastmetele (rabalaugastest servamäreni) iseloomulikud suurselgrootute kooslused.

Põhjavee mõju vähenemise ja toitainete suurenemise mõjusid liikide esinemisele uuriti võrreldes väheharjasusside (*Oligochaeta*) kooslusi Eesti looduslikes rabades ja Hollandi jäänukrabades (**5. osas**). Looduslikes rabades esines koosluste paiknemismustris selged seaduspärasused. Vähetoitelistes rabalaugastes esines vaid üks happelist keskkonda taluv mittesugulisel teel paljunev väheharjasussi liik. Eestis määras väheharjasusside levikut rabas sooveekogude esinemine, kus turba lagunemise tõttu oli troofsusaste kõrgem. Võrreldes väheharjasusside esinemist Eesti ja Hollandi rabades selgus, et liigid reageerivad vee toitainete sisalduse suurenemisele erinevalt tulenevalt nende toidusedelist. Hollandi rabades ei limiteerinud toitainete sisaldus väheharjasusside esinemist. Kõige liigirikkamaks osaks looduslikes rabades osutus servamäre (lagg). Enamik looduslikele servamäredele iseloomulikke liike Hollandi jäänukrabades ei esinenud, sest need osas soomaastikust on juba ammu kuivendatud ja kasutuses põllumajandusliku maana. Väheharjasusside esinemine Hollandi rabades kajastas nii vähetoiteliste rabaosade degradeerumist kui raba servale iseloomulike siirdesoo tingimuste hävimist.

Looduslikud rabalaukad on erakordselt toitainetevaesed ja rikkad humiainete poolest, mis takistavad esmast produktsiooni. Selgrootute toiduahela uurimiseks rabalaugastes määrati stabilisete isotoopide sisaldus esmases produktsioonis, surnud orgaanilises materjalis ja selgrootutes ning samuti nende objektide stabiilsete süsinikisotoopide sisaldus fosfolipiid rasvhappetes (PLFA) looduslikus rabalaukas Eestis (**6. osas**). Stabiilsete isotoopide sisaldus näitab eritevate troofiliste tasemete esinemist ja selgrootute põhitoidu päritolu nii erinevatel liikidel kui ka liigi erinevatel isenditel. Karnivoorsed ja omnivoorsed selgrootud omastavad

Kokkuvõte

küllastumata rasvhappeid (PUFA), mis pärinevad vetikatest ja tõenäoselt ka makrofüütidest, kui ka rasvhappeid, mida toodavad metaani tootvad bakterid (MOB). Oluline osa bakterite biomassist, mida kasutavad kõrgemad troofilised tasemed, pärineb tõenäoselt metaani tootvatelt bakteritelt. Ainuraksete ja zooplanktoni poolt sünteesitud küllastumata rasvhappeid (PUFA) kasutatakse sageli biomarkeriteks, mis näitavad vetikate osa toiduahelas. Lauka avaveeline zooplankton näib rohkem toituvat bakteritest aga putukatele ja fütoplanktonile on tähtsamad vetikad. Selgrootute küllastumata rasvhapete $\delta^{13}\text{C}$ väärtus viitab sellele, et toiduks kasutatavad vetikad saavad süsiniku metaani bakteritest. Seetõttu ei näita $\delta^{13}\text{C}$ väärtused selgrootutes otsest seost bakteritega vaid vetikad on toiduahela vaheastmeks. Hollandi rabade selgrootutes mõõdetud stabiilsete isotoopide väärtused näitavad, et raba toitainetega rikastumine muudab baassüsiniku allikat. Suureneb vetikatest ja surnud orgaanilisest materjalist päritoleva süsiniku osatähtsus ning metaanibakterite toodetud süsiniku osa toiduahelas väheneb.

Kokkuvõttes (**7. osa**) käsitletakse taastamisvõtete tõhusust ning elupaikade degradeerumise ning taastamise mõju veesalgrootute kooslustele rabamaastikes. Rabamaastiku taastamise puhul on edaspidi vajalik:

- Hollandi jäänukrabades suursalgrootute koosluste taastamiseks on vajalik vähendada õhusaaste poolt tingitud lämmastiku ja fosfor ühendite koormust.
- Raba taasatamisel on vajalik tagada looduslikule kompleksile iseloomulike erineva troofsusastmetega muutusastmete taastumine. Võimalusi selleks leiab soo puhvertsoonidesse jäävatel jäänukrabaga piirnevatel mahajäetud põllumajandusmaadel kui ka madalsoode kaitseks loodud aladel, kus leidub rabastuvaid laike.
- Vajalik on kaitsta tüüpiliste liikide reliktsid asaurkondi jäänukrabades, madalsoo kaitsealadel ja kanarbikunõmmede veekogudes.
- Edaspidi on vajalik keskenduda uuringutele, mis selgitavad käesolevas uuringus selgunud liikide reageeringute mehhanisme ning hindavad käesolevates teesides soovitatud kaitsekorralduslikke soovitusi rakendamist.



Dankwoord

◀ Jan Kuper collects aquatic invertebrates in a raised bog pool near Mukhrino Field Station, Western Siberia.

Dankwoord

Veel mensen hebben hun bijdrage geleverd aan het tot stand komen van dit proefschrift. Graag sta ik daar op deze pagina's bij stil. Het begon vele jaren voordat aan een proefschrift werd gedacht. Mijn interesse in de natuur werd aangewakkerd door Oom Ben die mij in mijn lagereschooltijd samen met mijn broer Alexander - en soms ook mijn zus Heleen - meenam naar de Dordtse Biesbosch en later ook andere gebieden om naar vogels en planten te kijken. Op de middelbare school boeiden de lessen van mijn biologieleeraar Jongeneel mij zeer. Toen ik begin 1997 voor mijn biologiestudie zocht naar een tweede stageonderwerp, bracht Jacques van Alphen me in contact met Hans Esselink †. De eerste ontmoeting met Hans in de anderhalve kamer die Stichting Bargerveen toen bezette in het IBN-kantoor te Arnhem bleek tekenend, zo ontdekte ik later. Jan Kuper vertelde me eerst van alles over het onderzoek aan de Grauwe Klauwieren. Hans moest eerst iets anders afmaken, had aansluitend alle tijd om mij in te wijden in het boeiende onderzoek en bracht me in een oude Citroën naar het station. Ik ruik de sigarenlucht nog. Gedurende de meer dan tien jaren die we hebben samen gewerkt, heb ik heel veel geleerd. Hans' bevologenheid, inzichten (niet alleen in ecologie en natuurbeheer!) en kritische houding hebben een blijvende invloed op mijzelf en het onderzoek. Ik ben blij met het vertrouwen dat hij mij gaf, de ruimte die ik kreeg om het onderzoek naar de fauna van hoogvenen uit te voeren en zijn stimulans om goed buiten de grenzen van ons land te kijken.

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Veel studenten droegen in de afgelopen jaren een grotere of kleinere steen bij aan het onderzoek. Hun namen zijn vermeld in de dankwoorden bij de verschillende voorgaande hoofdstukken. Allen dank voor jullie inzet, ideeën en het stellen van vragen. Mijn eerste student Sandra Lomans bedank ik bovendien voor de door haar afgedankte grote groene rugzak; die wordt nog steeds gebruikt voor het sjouwen van macrofaunamonsters en gereedschappen bij veldwerk!

De afdeling Milieukunde bood vanaf 1998 gastvrijheid aan de medewerkers van Stichting Bargerveen. Piet Nienhuis dank ik voor het bieden van deze mogelijkheid en de kritische opmerkingen en adviezen die hij als aanvankelijk beoogde promotor gedurende de eerste jaren van mijn onderzoek gaf. De prioriteiten lagen bij mij vaak bij andere projecten dan het schrijven van mijn proefschrift, zodat dit nog lang niet klaar was toen Piet met emeritaat ging. Jan Hendriks, bedankt voor het overnemen van de rol van promotor en de gelegenheid die jij mij gaf

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Voor het sorteren van monsters, determinaties en chemische analyses kon steeds gebruik worden gemaakt van de laboratoriumruimte en faciliteiten van de afdeling Ecologie en het Gemeenschappelijk Instrumentarium. Marij Orbons en Jelle Eygensteyn, dank voor jullie assistentie! Ook mijn consortiumgenoten Hilde Tomassen, Juul Limpens, Fons Smolders, Sake van der Schaaf, Geert van Wirdum en Jan Roelofs dank ik voor de prettige samenwerking, de discussies en het gezamenlijke veldwerk. De leden van het OBN-deskundigenteam Hoogvenen – opgevolgd door het deskundigenteam Nat Zandlandschap – dank ik voor de begeleiding van de verschillende fasen van het onderzoek.

Verder noem ik graag de volgende mensen, vanwege hun adviezen bij de start van het onderzoek, de determinatie van macrofauna, of de controle van onze determinaties: Bert Higler †, Piet Verdonchot, Henk van der Hammen †, Dik Hermes, Tjeerd-Harm van den Hoek, Klaas-Douwe Dijkstra, Vincent Kalkman, Kees Goudsmits, Frits Bink en Tarmo Timm. Henk Moller Pillot dank ik in het bijzonder voor zijn enthousiaste en deskundige bijdragen, hulp bij determinaties en de gezamenlijke excursies naar Denemarken en Wit-Rusland.

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Curriculum Vitae

◀ *Sphagnum* mosses are ecosystem engineers in mire ecosystems. Different *Sphagnum* species grow in different conditions. *Sphagnum riparium* grows in sites enriched for instance by percolating water like here in a spruce mire in Seitsemien national park, Finland. In The Netherlands this species is very rare and present in some fens and a hand peat cutting pit in the bog remnant Mariapeel.

Curriculum Vitae

Op 5 januari 1974 werd ik geboren te Dordrecht. In 1993 begon ik met mijn studie Biologie aan de Rijksuniversiteit te Leiden. Vanwege mijn interesse in insecten en relaties met hun omgeving koos ik voor een onderzoeksstage bij de afdeling Dierecologie van de Rijksuniversiteit Leiden. Door middel van laboratoriumexperimenten stelde ik vast welke natuurlijke voedselbronnen benut worden door zes soorten volwassen sluipwespen die parasiteren op verschillende fruitvliegsoorten. Mijn tweede onderzoekstage deed ik bij de Stichting Bargerveen, waarbij de vraag centraal stond of verslechtering van het insectenaanbod de oorzaak was voor het verdwijnen van Grauwe Klauwieren uit de Nederlandse duinen. In 1998 mocht ik mijn bul 'cum laude' in ontvangst nemen en kon ik bij de Stichting Bargerveen voor vier jaar aan de slag als junior onderzoeker met onderzoek naar randvoorwaarden voor het herstel van hoogvenen in het kader van het programma Overlevingsplan Bos en Natuur (later Ontwikkeling en Beheer Natuurkwaliteit) van het toenmalige Ministerie van Landbouw, Natuurbeheer en Visserij. Dit was een samenwerkingsproject met de afdeling Aquatische Ecologie en Milieubiologie van de toenmalige Katholieke Universiteit Nijmegen (thans Radboud Universiteit Nijmegen), de Wageningen Universiteit en NITG-TNO. Binnen dit project was het mijn taak de effecten van aantastingen en herstelmaatregelen op de watermacrofauna te onderzoeken met als doel aanbevelingen te genereren voor het herstelbeheer in de Nederlandse hoogveenrestanten. Dit onderzoek vormt de basis van dit proefschrift, dat ik tevens als (gast)medewerker bij de afdeling Milieukunde van de Radboud Universiteit Nijmegen heb afgerond.

Na voltooiing van de eerste fase van dit OBN-onderzoek volgden een tweede onderzoeksfase en onderzoeksprojecten in het Wierdense Veld, Bargerveen, Engbertsdijkswenen, Mariapeel en Deurnese Peel om de uitgangssituatie voorafgaand aan herstelmaatregelen vast te leggen, uitgevoerde maatregelen te evalueren en te adviseren over het herstelbeheer. Als onderzoeker en projectcoördinator werkte ik aan OBN-onderzoek naar herstel van biodiversiteit in het natte zandlandschap, een Europees LIFE Coop project gericht op het uitwisselen van praktische ervaring en wetenschappelijke kennis over herstelbeheer in hoogvenen en kustduinen tussen beheerders en onderzoekers, de samenstelling van teksten voor de website Natuurkennis.nl en uitwerking van de leefgebiedenbenadering van de zandgronden voor de provincie Noord-Brabant. Ook verzorgde ik excursies in Duitsland, Denemarken, Estland en West-Siberië voor terreinbeheerders en onderzoekers die betrokken zijn bij herstel van veenlandschappen. Vanaf het plotselinge overlijden van Hans Esselink op 30 augustus 2008 maak ik ook deel uit van het managementteam van de Stichting Bargerveen. Verder ben ik lid van het OBN-deskundigenteam Nat Zandlandschap. Ik werk graag samen met terreinbeheerders en collega-onderzoekers aan het verkrijgen en toepassen van de kennis die nodig is voor het behoud van biodiversiteit in Nederland en buitenland.

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Cover: Pool and *Sphagnum* dominated hummocks in the pristine raised bog of Nigula nature reserve, Estonia. Backside from top to bottom: 1) Meadow brown *Maniola jurtina* caught by sundew; 2) Albert Dees collecting aquatic invertebrates in bog remnant Engbertsdijkswenen, The Netherlands; 3) Adult water beetle *Laccophilus poecilus*; 4) Decaying birch trunks; 5) Adult dragonfly *Leucorrhinia albifrons*; (Photo of *Laccophilus poecilus*: René Krekels. Other photos: Gert-Jan van Duinen.)

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Het samenwerkingsverband tussen Natuurplaza en de Radboud Universiteit Nijmegen (IWWR) ontwikkelt, bundelt en verspreidt kennis op het gebied van herstel en behoud van biodiversiteit en ecosystemen.

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- Vereniging SOVON vogelonderzoek Nederland
- Stichting reptielen, amfibieën en vissenonderzoek Nederland (RAVON)
- Stichting floristisch onderzoek Nederland (FLORON)
- Zoogdiervereniging
- Vereniging onderzoek flora en fauna (VOFF)

Deze combinatie van organisaties verbindt het verspreidingsonderzoek met het wetenschappelijk onderzoek. De koppeling van veldwaarnemingen in ruimte en tijd met ecologische lab-, veld- en beheerexperimenten resulteert in innovatieve kennis. De Natuurplaza partners hebben een breed en actief netwerk van vrijwilligers. Hierdoor is er een continue vernieuwing, ontwikkeling en doorstroom van kennis, waardoor maatschappelijke vraagstukken snel en adequaat aangepakt kunnen worden.

Stichting Bargerveen heeft als doel het ontwikkelen van kennis voor systeemgericht natuurherstel met focus op fauna en het (inter)nationaal verspreiden van kennis. Daarnaast is het bijdragen aan de opleiding van studenten en junior onderzoekers een belangrijke doelstelling. De onderzoeksvragen die de stichting beantwoordt komen voort uit de problemen waar beheerders of beleidsmakers tegenaan lopen. Veel thema's zijn gerelateerd aan de effectiviteit van de beheersmaatregelen, alsmede aan de VER-thema's, zoals verzuring, vermesting en verdroging. Het onderzoek wordt uitgevoerd in binnen- en buitenland en in een scala van landschappen, waaronder de kustduinen, stuifzanden, kalkgraslanden, laagveenwateren, hoogvenen, heide en vennen.

Stichting Bargerveen is met de andere Natuurplaza partners gehuisvest binnen de Radboud Universiteit Nijmegen en werkt nauw samen met de afdelingen dierecologie en -ecofysiologie, milieukunde, aquatische ecologie en milieubiologie.



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