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

G.A. van Duinen[1,2,3], K. Vermonden[1,3], P.L.E. Bodelier[4], A.J. Hendriks[3], R.S.E.W. Leuven[3], J.J. Middelburg[5,6], G. van der Velde[2,7] & W.C.E.P. Verberk[1,2]

[1] Bargerveen Foundation / Radboud University Nijmegen, P.O. Box 9010, 6500 GL Nijmegen, The Netherlands

[2] Radboud University Nijmegen, Institute for Water and Wetland Research, Department of Animal Ecology and Ecophysiology, P.O. Box 9010, 6500 GL Nijmegen, The Netherlands

[3] Radboud University Nijmegen, Institute for Water and Wetland Research, Department of Environmental Science, P.O. Box 9010, 6500 GL Nijmegen, The Netherlands

[4] Netherlands Institute of Ecology (NIOO-KNAW), Department of Microbial Ecology, P.O. Box 50, 6700 AB Wageningen, The Netherlands

[5] Utrecht University, Faculty of Geosciences, Department of Earth Sciences - Geochemistry, P.O. Box 80.021, 3508 TA Utrecht, The Netherlands

[6] Department of Ecosystem Studies, Royal Netherlands Institute for Sea Research (NIOZ), Korringaweg 7, 4401 NT Yerseke, The Netherlands

[7] Naturalis Biodiversity Center, P.O. Box 9517, 2300 RA Leiden, The Netherlands

E-mail: G.vanDuinen@science.ru.nl

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 $\delta^{13}C$ values of PUFAs in invertebrates point to the use of algae that possibly derived carbon from MOB. Therefore, depleted $\delta^{13}C$ values of invertebrates do not necessarily implicate a direct pathway between MOB and these invertebrates, but algal food sources forming an intermediate level.

Keywords: peatland, algae, methane oxidizing bacteria, zooplankton, insects, stable isotopes, fatty acids

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 and Lancaster 2002), harbouring a large biodiversity of aquatic macroinvertebrates (Desrochers and 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 and Jeglum (2006) suggested bacteria feeding on dissolved humic substances as a second basal food source, in addition to photosynthesis. Jones (1992)

72 described humic substances as an important carbon source in planktonic food chains in lakes
73 in which primary production of algae is limited by oligotrophy or humic substances. Although
74 humic substances are highly recalcitrant to microbial degradation, Tranvik (1988) found that
75 lakes with a high content of humic substances could support a higher bacterial biomass than
76 clearwater lakes due to their larger pools of dissolved organic carbon (DOC).
77 Biogenic methane could be a third basal carbon source. In bogs methane is produced
78 during the decomposition of peat (Raghoebarsing et al. 2005). Methane-derived carbon can
79 contribute to the food web via methanotrophic bacteria, which are found to be ingested by
80 zooplankton (Bastviken et al. 2003, Taipale et al. 2007), chironomid larvae (Jones et al.
81 2008), and caddisfly larvae grazing their own cases (Trimmer et al. 2009).
82 A powerful tool to distinguish between the potential food sources and to determine the
83 configuration of food webs is dual stable isotope analysis ($\delta^{13}C$ and $\delta^{15}N$) of producers and
84 consumers. This approach is based on a predictable change in the natural abundance stable
85 isotopes composition between the different trophic levels (DeNiro and Epstein 1978,
86 Minagawa and Wada 1984) and has been applied in a wide range of ecosystems. However,
87 the extent to which the pathways conveying organic matter to consumers and their predators
88 can be inferred solely from stable isotopic signatures depends on the variation and
89 distinctness of the isotopic signatures of basal food sources. In addition, the isotopic signature
90 of a consumer can result from the consumption of a single food source, but more realistically
91 from the consumption of a mixture of two or more food sources. One way to gain a better
92 understanding of the relative importance of basal food sources in food webs is to combine the
93 analysis of natural abundance stable isotopes composition with analyses of phospholipid-
94 derived fatty acids (PLFAs) composition (Kharlamenko et al. 2001, Perga et al. 2006, Van
95 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 $\delta^{13}C$ value (Boschker and Middelburg 2002). The role of methane in freshwater food webs has recently attracted much attention (Jones and 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 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?

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 (Fig. 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, 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).

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 mesh size 45 μm. Zooplankton was collected from the open water by means of a plankton net with mesh size 115 μm 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 (30 x 25 cm) 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^{o}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 $\delta^{13}C$ value of DOC were collected in December 2006 and July 2007 by filtering surface water over a filter with mesh size 0.2 µm (Schleicher & Schuell FP 030/3) and adding 100 µL 50% H_3PO_4 to 40 mL water sample. The carbon isotopic 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 CO_2 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:

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

where $R = {}^{13}C/{}^{12}C$ or ${}^{15}N/{}^{14}N$. $\delta^{13}C$ or $\delta^{15}N$ is the per mille (‰) deviation of the sample from their isotope standards, that are Vienna PeeDee belemnite for $\delta^{13}C$ and atmospheric N_2 for

$\delta^{15}N$. Average reproducibility based on replicate measurements of samples and internal standards Sucrose (IAEA-CH-6) for $\delta^{13}C$ and Ammonium sulphate (IAEA-N-2) for $\delta^{15}N$ 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 $\delta^{13}C$ 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 and 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 $\delta^{13}C$ value was calculated and used as input to the mixing model. We used IsoSource version 1.3.1 (Phillips and 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 $\delta^{13}C$ 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 Finnagan TRACE GC-MS system. For identification of methanotroph-specific PLFA, extracts of cultures of *Methylomonas methanica S1* NCIMB 11130, *Methylomicrobium 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 ωxc or ωxt, 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 10^{th} 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 Finnagan TRACE GC) equipped with a polar capillary column (SGE, BPX-70; 50 m by 0.32 mm by

225 0.25 μm), using the following oven conditions: initial temperature of 50 °C for 1 min, and
226 then the temperature was programmed to 130°C using a ramp of 40 °C min^{-1} followed by an
227 increase to 230 °C with a ramp of 3 °C min^{-1}.
228 Stable carbon isotope ratios for individual FAME were determined using a Varian
229 3400 GC equipped with an ATAS Optic 2 programmable direct thermal desorption injection
230 system. The GC was coupled via a type II combustion interface to a Finnigan Delta S isotope
231 ratio mass spectrometer. The same polar capillary column was used as for FAME
232 identification and quantification on the GC-FID and GC-MS systems. The oven temperature
233 for the GC-isotope ratio mass spectrometry analyses was as follows: initial temperature of
234 50°C for 4 min, and then the temperature was programmed to 130°C using a ramp of 30°C
235 min^{-1}, which was immediately followed by an increase to 200°C using a ramp of 6°C min^{-1}, a
236 subsequent increase to 220°C using a ramp of 5°C min^{-1}, and a final increase to 250°C using a
237 ramp of 20°C min^{-1}. The sample was injected into the direct thermal desorption system at
238 50°C, after which the temperature was programmed to 260°C with a ramp of 10°C s^{-1}.
239 PLFAs with a relative concentration < 0.1% are disregarded. $\delta^{13}C$ values of PLFAs
240 with a relative concentration < 1% are regarded as unreliable and not presented here. The
241 potential affiliated biota of the PLFAs found in SOM and invertebrates was taken from
242 Boschker and Middelburg (2002), Dijkman and Kromkamp (2006) and Taipale et al. (2009),
243 accomplished with various other papers mentioned in the results and discussion sections.
244

Results

Stable isotopic signatures

The various invertebrate species collected in the three bog pools differed in their $\delta^{13}C$ values (Table 2), indicating a differential use of basal carbon sources, and in their $\delta^{15}N$ values, indicating the presence of multiple trophic levels (Fig. 2). The living and dead plant material showed the lowest $\delta^{15}N$ values. Most aquatic invertebrate species collected were carnivorous according to literature (Table 2). The highest $\delta^{15}N$ 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 $\delta^{15}N$ 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 $\delta^{15}N$ values were found among the invertebrate species known as herbivores, herbi-detritivores or omnivores, e.g. zooplankton in pool N1 (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 matter, and DOC (Fig. 2). Periphyton (mainly consisting of green algae) was the most depleted potential basal carbon source found. The periphyton varied considerably in their $\delta^{13}C$

values between the three bog pools (Table 2). The lowest $\delta^{13}C$ value of periphyton, found in N3 (-34.69‰), could account for the $\delta^{13}C$ value of at least the more depleted half of the invertebrate food web, assuming an enrichment (less negative) of 0 to 1‰ for the $\delta^{13}C$ 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 $\delta^{13}C$ was more depleted than those of the potential basal carbon sources.

The $\delta^{13}C$ 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 $\delta^{15}N$ 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 $\delta^{15}N$ values (according to Minagawa and Wada (1984) and Post (2002) and confirmed by the average $\delta^{15}N$ values of the trophic levels in this study (Fig. 2)) and of 0 to 1‰ for the $\delta^{13}C$ 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: 16:1ω8c and 16:1ω5t; Nichols et al. 1985, Bowman et al. 1993) were found in SOM and all invertebrates (Table 5), but at low concentrations. The $\delta^{13}C$ 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 18:1ω8c, characteristic for MOB II (Bodelier et al. 2009), was found in SOM and had a

295 $\delta^{13}C$ value of -38.0‰ (Table 6). This PLFA was not detected in the invertebrates. Here, it
296 must be noted that in the PLFA analysis of the invertebrates the high peak of the PLFA
297 18:1ω9c may have hidden the peak of the PLFA 18:1ω8c (Deines et al. 2007). The PLFA
298 18:1ω9c was present in high concentration in all invertebrates and in a lower concentration in
299 SOM. In addition, PLFAs 18:2ω6c,12c and 18:2ω7c,12c, that are diagnostic biomarkers of
300 *Methylocystis* strains (MOB II) according to Bodelier et al. (2009), were detected in SOM and
301 two insect species.
302 Methyl-branched and branched unsaturated PLFAs that are typical for sulphate-
303 reducing Bacteria and Actinomycetes (Kroppenstedt 1992, O'Leary and Wilkinson 1988)
304 were found at low concentrations in SOM and occasionally in invertebrates. PLFAs with
305 cyclopropyl rings and branched PLFAs that are typical for bacteria (O'Leary and Wilkinson
306 1988, Zelles 1999) had a substantial concentration in SOM, but were found in low
307 concentrations in the invertebrates. The $\delta^{13}C$ values of these PLFAs varied between -38.5 and
308 -29.9‰. The monounsaturated PLFA 18:1ω7c, typical for bacteria (Wilkinson 1988) and a
309 major PLFA in MOB (Bodelier et al. 2009), but also found in low abundance in various
310 groups of algae (Dijkman and Kromkamp 2006), was the most abundant PLFA in SOM and
311 present in all invertebrates and had $\delta^{13}C$ values between -35.9 and -29.8‰. The total relative
312 concentration of the above mentioned PLFAs typical for bacteria was 57.1% in SOM, 11.4%
313 in zooplankton, and between 2.3% and 10.4% in the insect species.
314 The total relative concentration of polyunsaturated PLFAs (PUFAs) was 4.5% in SOM
315 and 5.2% in zooplankton, but between 36.3% and 52.6% in the insects. The PLFAs 18:3ω6,
316 20:4ω?, and 20:5ω3 were found in considerable amounts in all insects analysed here. The
317 PLFA 18:2ω6c,9c was present in high concentration only in the predators *Notonecta lutea*,
318 *Ilybius aenescens* and *Ilybius guttiger* and (almost) absent in the other invertebrates and SOM.
319 The PLFA 18:3ω6 is reported from algae (Chrysophyceae) and Cyanobacteria (Taipale et al.

2009 and references therein) and fungi (Desvilettes et al. 1997). The PLFA 20:4ω6 is found in minor amounts in some Bacillariophyceae (diatoms), but in higher abundance in Rhodophyta (Dijkman and Kromkamp 2006). The PLFAs 20:4ω6c and 18:3ω6c are both produced by Protozoans grazing on MOB in wet soils (Murase et al. 2011). The PLFA 20:5ω3 is typical for various groups of algae, including diatoms and Cryptophyta (Dijkman and Kromkamp 2006). The PLFA 18:2ω6 is used as biomarker for fungi (Frostegård and Bååth 1996, Desvilettes 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 and Kromkamp 2006) and in Cyanobacteria (Caramujo et al. 2008).

PUFAs had the most depleted $\delta^{13}C$ values among all PLFAs found. The PUFA 20:4 was the most depleted PLFA in SOM and zooplankton with $\delta^{13}C$ values of -45.5‰ and -47.1‰, respectively, but less depleted in the insects, with $\delta^{13}C$ values between -40.3 and -33.2‰. In contrast to the PLFA 20:4, the PLFA 20:5ω3 was more enriched in ^{13}C in SOM than in the invertebrates. In most insects the PUFA 18:3ω6 was the most depleted PLFA with $\delta^{13}C$ values between -42.3 and -37.2‰. This PUFA was not detected in zooplankton and had a low concentration in SOM. Therefore no reliable $\delta^{13}C$ value could be obtained for 18:3ω6 in SOM.

Among the monounsaturated PLFAs (MUFAs), 18:1ω9c 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 and Kromkamp 2006) and Cyanobacteria (Caramujo et al. 2008) as well. The high relative concentration of 18:1ω9c in the insects indicates the ingestion of algae by these insects or by their prey. However, 18:1ω9 is also a major PLFA of methanotrophs in wet peat soils (Chen et al. 2008). In zooplankton the relative concentration of 18:1ω9c was with 10.6% lower than

345 in the insects, but also the highest among the MUFAs, followed by 16:1ω7c (10.0%) and
346 18:1ω7c (7.2%). The PLFA 16:1ω7c is a major PLFA in methanotrophic bacteria, but also in
347 green sulphur bacteria (Taipale et al. 2009 and references therein), nitrifiers (De Bie et al.
348 2002), and diatoms (Dijkman and Kromkamp 2006).
349 The total relative concentration of saturated PLFAs was between 16.3% and 30.5% in
350 SOM and insects, but 60.6% in zooplankton, with 41.4% consisting of the PLFA 16:0. This
351 PLFA is also abundant in several groups of algae and bacteria (Taipale et al. 2009 and
352 references therein).
353

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 (Fig. 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 $\delta^{13}C$ values > -28‰. The $\delta^{13}C$ 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 half the invertebrate food web more depleted in ^{13}C. The $\delta^{13}C$ values of periphyton varied considerably between the pools. In pool N1 it had a $\delta^{13}C$ value of -27.4‰. Possibly, periphyton, or periphyton components, with a $\delta^{13}C$ value < -30‰ were also present in N1, like in N2 and N3. In addition, the $\delta^{13}C$ values of phytoplankton can be lower than -30‰ (Taipale et al. 2007). The variation in $\delta^{13}C$ 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 $\delta^{13}C$ 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 $\delta^{13}C$ 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

379 sustaining the more depleted half of the invertebrate food web in all three bog pools sampled
380 here.
381 The stable isotopic signatures of the invertebrates and potential basal food sources
382 alone do not resolve the importance of living or dead material of *Sphagnum* mosses and
383 vascular plants, or particulate and dissolved organic matter originating from the peat in which
384 the pools are embedded, as basal food source for the invertebrates with $\delta^{13}C$ values > -28‰.
385 The range in $\delta^{13}C$ values of the algae samples collected here (periphyton and larger algae)
386 implies that the whole invertebrate community could be sustained only by different species of
387 algae. Alternatively, the carbon sources for the invertebrates could consist of a combination of
388 algae, living or dead organic material from macrophytes, dissolved organic carbon
389 compounds and bacteria and fungi living in or on the various organic substrates.
390
391 *Biomarker PLFAs and pathways in the food web*
392 The variation in the PLFA composition (Table 4) and in the $\delta^{13}C$ values of PLFAs
393 (Table 5) of the invertebrates indicates that they used different basal food sources. They
394 assimilated fatty acids that are specific for MOB, for other bacteria, as well as
395 polyunsaturated fatty acids (PUFAs) that are derived from algae, and maybe macrophytes,
396 either or not via fungi. These PLFA data confirm the importance of algae (periphyton and
397 possibly phytoplankton) inferred from the stable isotope data. The elucidation of the relative
398 importance of these basal food sources to the invertebrate food web of bog pools is however
399 somewhat constrained because many of the recorded PLFAs cannot unambiguously be
400 attributed to either macrophytes, algae, or methanotrophic or other bacteria. Furthermore,
401 eukaryotes other than algae or macrophytes might synthesize PUFAs that are used as
402 biomarkers for algae.

403 The PUFAs 20:5ω3, assumed to be characteristic for algae, as well as 20:4ω6, can be
404 produced by zooplankton from 18:3ω3 and 18:2ω6, respectively, although this ability differs
405 between groups (Caramujo et al. 2008 and references therein). According to Arts (1999) most
406 species of freshwater zooplankton cannot synthesize or elongate PUFAs with 18 or 20 C-
407 atoms and must obtain them from their diet. This inability is also found for omnivorous
408 caddisfly larvae and the PUFAs 18:2ω6, 18:3ω3, 20:4ω6, and 20:5ω3 (Torres-Ruiz et al.
409 2010). This finding may therefore be extended for the invertebrate species studied here.
410 Murase *et al*. (2010) found that the PLFAs 18:3ω6 and 20:4ω6 were produced by protozoans
411 grazing on MOB. It is unknown if this pathway is important in bog pools. However, it is
412 unlikely to be the only pathway conveying these PLFAs to the insects, as the amounts of
413 18:3ω6, 20:4ω?, 20:5ω3, and their possible ω6 and ω3 precursors in SOM and zooplankton
414 are small compared to the high amounts of these PUFAs in the insects (Table 5), although
415 preferential assimilation of PUFAs by the insects might result in a higher relative amount of
416 PUFAs in the insects than in their food. Moreover, the abundance of green algae and the
417 presence of some pieces of *Sphagnum* mosses and other macrophytes in the guts of several
418 insects in the pools studied here (Odonata nymphs and larvae of Chironomidae and
419 Trichoptera; personal observations) support the conclusion that algae are indeed an important
420 basal food source in the bog pool food web, next to bacteria and possibly macrophytes,
421 especially for the insects that all showed a high concentration of PUFAs (Fig. 3).
422 The amount of 20:5ω3, characteristic for diatoms and also present in some other
423 groups of algae, but absent in bacteria, might help to roughly estimate the relative amount of
424 PLFAs originating from either bacteria or algae in SOM and invertebrates, when a more or
425 less constant ratio between 20:5ω3 and the other PLFAs in the algal community is assumed.
426 The relative amount of the PLFA 20:5ω3 in zooplankton was six times higher than in SOM,
427 and in insects it was fourteen to thirty-seven times higher than in SOM (Table 5). Thus, most

428 of the PLFA content in SOM was present in the bacterial community, and possibly partly in
429 protozoa like flagellates and ciliates that ingested them. In the invertebrates much larger parts
430 of the PLFA content originated from algae, but the insects seem to rely more on algae than
431 pelagic zooplankton does (Fig. 3).

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

452 The PLFA composition of the collected SOM shows that the living biomass in SOM is
453 dominated by bacteria. In a wide range of bacterial dominated sediments the sum of the
454 relative amounts of the PLFAs i14:0, a15:0, i15:0, i16:0, and 18:1ω7c, characteristic for
455 bacteria, is 28±4% (Middelburg et al. 2000). This sum was much higher in the SOM of the
456 bog pools studied here (41.3%), due to the high abundance of 18:1ω7c (31%). The PLFA
457 18:1ω7c is likely to be the prevailing lipid in methanotrophs in *Sphagnum* moss (Bodelier et
458 al. 2009, Van Winden et al. 2010). Using the relative amount of the MOB specific PLFAs
459 16:1ω8c and 18:1ω8c in SOM and the fairly constant ratio between these specific PLFAs and
460 non-specific PLFAs found in MOB strains (Bodelier et al. 2009), we may assume the MOB to
461 make up about 10% of the bacterial population in the SOM.
462
463 *A pathway of methane to invertebrates via algae?*
464 As methane and MOB are depleted in ^{13}C, the $\delta^{13}C$ values of invertebrates
465 assimilating methane-derived carbon are similarly depleted (Taipale et al. 2007 and 2009).
466 The low $\delta^{13}C$ values of the zooplankton samples compared to most insects, including all
467 insects of low trophic level (Table 2), indeed corresponds to the larger reliance of
468 zooplankton on MOB inferred from the PLFA data. However, overall, the PUFAs
469 characteristic for algae or other plants (18:3ω6, 20:4ω?, 20:5ω3) were more depleted than the
470 PLFAs typical for bacteria, including the PLFAs typical for MOB. For some of these PUFAs
471 this could be explained by the possibility that they can also be synthesized by protozoans (c.f.
472 Murase et al. 2010) or zooplankton (c.f. Caramujo et al. 2008). The PLFA 20:4ω? was much
473 more depleted in ^{13}C in the zooplankton than in the insects, indicating a difference in carbon
474 pathways. As methane is known to be depleted in ^{13}C, this would suggest that the zooplankton
475 synthesized this PLFA from precursor fatty acids (c.f. Caramujo et al. 2008) ingested via

476 MOB, or that they ingested protozoa that synthesized this PLFA (c.f. Murase et al. 2010),
477 whereas the insects might get the PLFA 20:4ω? via algae and herbivorous prey.
478 It is, however, remarkable that in zooplankton the $\delta^{13}C$ values of the PLFAs 16:1ω7c,
479 presumably derived from MOB, as suggested above, and 18:1ω7c, derived from MOB and
480 other bacteria, were also generally less depleted than in the insects (Table 6). As methane is
481 depleted in ^{13}C, the $\delta^{13}C$ values of the PLFAs in MOB are expected to be lower than those in
482 algae, assuming the latter use CO_2 for photosynthesis. However, Raghoebarsing et al. (2005)
483 showed that submerged *Sphagnum* mosses can use CH_4 as carbon source, converted to CO_2
484 via endosymbiontic MOB. Could also the algae in bog pools obtain methane-derived CO_2 via
485 MOB living as endosymbionts or as a constituent of the periphyton (or biofilm), explaining
486 the relatively depleted $\delta^{13}C$ values of algae-derived PUFAs in the insects? Labelling studies
487 with ^{13}C-bicarbonate or $^{13}CH_4$ (Raghoebarsing et al. 2005, Deines et al. 2007, Pace et al.
488 2007) are required to verify the existence of such intriguing pathways from both MOB and
489 algae in the food web of bog pools.
490
491 *Combining the outcomes of stable isotopes and PLFA analyses*
492 The variation in the stable isotopic signatures (Table 2), the PLFA composition (Table
493 5), and the $\delta^{13}C$ values of PLFAs (Table 6) indicated that the invertebrates in bog pools use
494 different basal food sources. The $\delta^{13}C$ values of different potential basal food sources and
495 invertebrates indicated that algae (in periphyton and possibly phytoplankton) sustain at least
496 half the invertebrate food web. The PLFA composition showed that algae, MOB and other
497 bacteria are ingested by the invertebrates, directly or via their prey. Pelagic zooplankton
498 seems to rely more on bacteria, whereas for insects algae are more important. This variation in
499 relative importance of basal food sources is indicated in the schematic representation of the
500 food web (Fig. 3) with variation in the thickness of the black arrows. A considerable part of

501 the bacterial biomass conveyed to higher trophic levels in the bog pools likely originates from
502 MOB. The results suggest that algae in bog pools use methane derived carbon, possibly via
503 MOB (indicated with the grey curved arrows in Fig. 3). Invertebrates grazing on periphyton
504 likely ingest the MOB associated with the periphyton. Thus, depleted $\delta^{13}C$ values of whole
505 organisms, or PLFAs, do not necessarily implicate a direct pathway between MOB and these
506 organisms. Instead, algae could be an intermediate, constituting a major food source for
507 aquatic invertebrates.
508

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700 Table 1. Average (± standard deviation) quality data of surface water, sediment pore water
701 and sedimented organic matter at the sampling sites. N=2 sampling periods.
702
703
704 Table 2. Values (‰) of $\delta^{15}N$ and $\delta^{13}C$ of sedimented and dissolved organic matter, plants and
705 invertebrates of the three bog pools N1, N2 and N3. Invertebrates are arranged according to
706 trophic group indicated in column T (c = carnivore, d = detritivore, h = herbivore, hd = herbi-
707 detritivore, o = omnivore) and subsequently to taxonomical group indicated in column 'Tax.'
708 (Odo=Odonata, Het=Heteroptera, Col=Coleoptera, Meg=Megaloptera, Dip=Diptera,
709 Tri=Trichoptera, Ara=Aranaea, Cru=Crustacea, Eph=Ephemeroptera). In column # the
710 number 1 indicates that specimens of the species or higher taxon were analysed individually,
711 otherwise individuals were pooled per species or higher taxon.
712
713
714 Table 3. Values (‰) of $\delta^{15}N$ and $\delta^{13}C$ of invertebrates (imagines, unless otherwise indicated)
715 walking and flying around the bog pools. The taxa are arranged from low to high $\delta^{13}C$.
716
717
718 Table 4. Means and 1 and 99 percentiles of the feasible contribution of potential basal carbon
719 sources to the different trophic groups of invertebrates. The classification of invertebrate
720 species in the trophic groups is given in Table 2.
721
722
723 Table 5. PLFA composition, as percentage of total PLFA's, in sedimented organic matter
724 (SOM) and aquatic invertebrates.

725

726

727 Table 6. δ13C (‰) of PLFAs in sedimented organic matter (SOM) and aquatic invertebrates.

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729

730 Figure 1. Geographical location of the three raised bog pools studied in Nigula Nature

731 Reserve, Southwest Estonia.

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733

734 Figure 2. Average values ± SE (‰) of $\delta^{15}N$ and $\delta^{13}C$ of different groups of primary

735 producers, sedimented organic matter (SOM), and different trophic groups of invertebrate

736 species in the bog pools N1, N2, and N3. The vertical lines indicates the average $\delta^{13}C$ (± SE)

737 of dissolved organic matter (DOC) of the three bog pools. The classification of invertebrates

738 in the different trophic groups is given in Table 2.

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740

741 Figure 3. Schematic representation of the food web in raised bog pools. The thickness of the

742 black arrows indicate the relative importance of that relation to the invertebrate group, as

743 derived form the results described in this paper. The grey curved arrows indicate the possible

744 role of methane oxidising bacteria (MOB) in the carbon supply to the primary producers.

745

Site	Nigula 1	Nigula 2	Nigula 3
Surface water			
pH	3.9±0.1	3.9±0.2	4.0±0.1
o-PO_4 (µmol/L)	0.23±0.10	0.17±0.16	0.28±0.01
NO_3+NH_4 (µmol/L)	4.8±2.5	9.3±4.0	10.7±9.0
Ca (µmol/L)	17.2±8.9	23.3±12.0	25.3±9.9
Cl (µmol/L)	58.7±5.3	90.5±31.3	71.3±5.9
Dissolved inorganic carbon (DIC) (µmol/L)	22.6±31.9	44.7±25.5	32.6±40.2
Dissolved organic carbon (DOC) (µmol/L)	1654±98	1925±59	1671±457
Sediment pore water			
pH	4.7±0.7	4.6±0.6	4.5±0.2
o-PO_4 (µmol/L)	0.18±0.06	0.16±0.11	0.47±0.65
NO_3+NH_4 (µmol/L)	2.2±2.6	12.7±14.5	26.6±17.6
Ca (µmol/L)	58.2±44.5	55.1±37.7	51.0±26.7
Cl (µmol/L)	55.2±8.9	58.5±6.9	63.0±18.3
Dissolved inorganic carbon (DIC) (µmol/L)	42.3±14.9	38.9±14.9	54.2±29.1
Sedimented organic matter			
C:P (g/g)	1293±636	1309±403	652±347
C:N (g/g)	29.8±17.7	24.7±15.1	17.2±5.7
Ca (µmol/gDW)	72.9±25.1	51.3±10.0	66.4±14.3

Species	#	Tax.	T	N1		N2		N3	
				$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$
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
Sphagnum cuspidatum (submerged)				-2.39	-26.73	-2.11	-26.58	1.22	-23.92
S. magellanicum (from lawn)				-3.35	-24.50	-4.03	-24.76	-5.14	-27.44
S. magellanicum (submerged)				-2.48	-24.53	-3.60	-24.59		
Carex limosa (living plant)						-5.72	-27.79	-0.87	-27.27
Carex rostrata (dead leaves)								-0.85	-26.58
Carex rostrata (water)								-0.35	-26.75
Carex rostrata (roots)								-3.17	-26.69
Scheuchzeria palustris						-6.06	-25.57	-2.65	-26.19
Rhynchospora alba				-3.55	-27.40				
Utricularia minor						-1.18	-22.51	-1.41	-21.06
Betula 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				
Anax imperator nymphs last stage		Odo	c			2.77	-29.62		
Anax imperator young nymphs		Odo	c			2.24	-28.09		
Aeshna spec. nymphs		Odo	c	1.01	-26.35			0.39	-27.87
Libellula spec. nymphe 1	1	Odo	c	0.87	-31.18			-0.44	-26.92
Libellula spec. nymphe 2	1	Odo	c	-0.31	-30.26			0.18	-27.77
Leucorrhinia spec. nymphs		Odo	c	0.18	-25.51				
Libellulidae nymphs		Odo	c	0.65	-30.12	0.48	-29.32	-0.77	-27.77
Cymatia bonsdorffii		Het	c	0.77	-30.97	1.47	-32.77	-0.38	-30.02
Notonecta glauca 1	1	Het	c	1.22	-28.52				
Notonecta glauca 2	1	Het	c	2.24	-28.62	2.67	-25.32		
Notonecta glauca 3	1	Het	c	10.33	-32.07	2.50	-28.91		
Notonecta glauca 4	1	Het	c	8.19	-34.70	2.29	-29.19	1.01	-28.05
Ilyocoris cimicoides 1	1	Het	c	0.73	-25.26	0.66	-28.21	-0.05	-24.58
Ilyocoris cimicoides 2	1	Het	c	0.46	-24.80				
Nepa cinerea	1	Het	c	4.68	-27.61				
Ranatra linearis	1	Het	c	4.15	-30.30	6.65	-26.18		
Gerris spec.		Het	c			1.76	-25.49		
Acilius canaliculatus 1	1	Col	c	2.26	-33.89	4.08	-29.93	2.41	-28.68
Acilius canaliculatus 2	1	Col	c	2.19	-28.85	3.67	-28.93		
Acilius canaliculatus 3	1	Col	c	3.41	-31.14				
Acilius canaliculatus 4	1	Col	c	2.24	-28.71				
Acilius canaliculatus 5	1	Col	c	6.31	-28.82				
Acilius canaliculatus 6	1	Col	c	3.13	-27.25				
Acilius sulcatus 1	1	Col	c	1.77	-30.33	2.21	-29.19		
Acilius sulcatus 2	1	Col	c	1.74	-29.77				
Acilius sulcatus 3	1	Col	c	2.04	-32.55				
Acilius sulcatus 4	1	Col	c	3.80	-30.75				
Acilius sulcatus 5	1	Col	c	4.28	-30.03				
Acilius sulcatus 6	1	Col	c	1.89	-31.22				
Dytiscus dimidiatus 1	1	Col	c	4.24	-29.00	5.33	-29.11		

Dytiscus dimidiatus 2	1	Col	c	5.50	-28.37	3.64	-30.60	2.63	-38.57
Dytiscus lapponicus 3	1	Col	c	1.97	-29.83	1.87	-29.24		
Dytiscus lapponicus 4	1	Col	c	0.94	-29.84	1.84	-29.12		
Dytiscus lapponicus 5	1	Col	c			1.84	-28.97		
Graphoderus cinereus		Col	c					0.62	-28.10
Hyphydrus ovatus		Col	c					0.23	-27.03
Ilybius subaeneus		Col	c	0.95	-31.24	0.35	-26.54		
Gyrinus spec.		Col	c	3.89	-32.47				
Dytiscidae larvae		Col	c					0.40	-24.33
Sialis spec. larvae		Meg	c					-1.13	-29.94
Chaoborus spec. larvae		Dip	c			2.51	-29.90		
Ablabesmyia spec. larvae		Dip	c					-0.33	-28.24
Polycentropodidae larvae		Tri	c	1.30	-27.93	0.59	-27.68	1.30	-28.19
Argyroneta aquatica (small)		Ara	c	2.67	-27.57	2.97	-25.81	2.10	-24.76
Argyroneta aquatica (large)		Ara	c			3.07	-28.91		
Hydracarina		Aca	c					3.78	-27.88
Asellus aquaticus		Cru	d					-1.22	-26.53
Leptophlebia vespertina nymphs		Eph	hd			-1.12	-28.70	-1.12	-25.94
Psectrocladius spec. larvae		Dip	hd	-1.56	-24.25				
Chironominae larvae		Dip	hd	0.56	-26.28				
Chironomus spec. larvae		Dip	hd					-2.04	-28.43
Phalacrocera replicata larvae		Dip	h			-0.37	-27.27	-0.12	-25.32
Sigara scotti		Het	o	0.48	-29.17			-1.40	-28.61
Sigara semistriata		Het	o					-0.45	-26.17
Hesperocorixa linnei		Het	o					2.04	-29.19
Hesperocorixa sahlbergi		Het	o	2.66	-30.24				
Glaenocorisa propinqua		Het	o	1.35	-31.20				
Corixa dentipes		Het	o	1.50	-30.33			-1.07	-30.63
Phryganea bipunctata larvae		Tri	o	0.07	-26.62			-0.39	-26.27
Zooplankton				-0.94	-33.27			2.81	-31.36

Species	**$\delta^{15}N$**	**$\delta^{13}C$**
Enallagma cyathigerum	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 *(Limnephilus* spec.)	1.22	-27.77
Tetragnatha spec.	2.37	-27.76
Phyralidae	2.96	-27.74
Lycosidae	1.91	-27.53
Lepidoptera (moth)	0.71	-27.23
Sympetrum danae	0.50	-27.00
Brachycera	4.33	-26.88
Diplopoda	-2.88	-26.86
Nabidae	-1.11	-26.77
Hippodomia spec.	0.04	-26.75
Aranaeidae	2.98	-26.56
Bombus jonellus	-0.59	-26.41
Trichoptera *(Limnephilus* spec.)	-1.04	-26.34
Pterostichus minor	-0.40	-26.24
Metrioptera brachyptera	-4.34	-26.19
Thomisidae	4.08	-26.00
Formica spec.	0.51	-25.73
Lepidoptera larva	-4.29	-25.43
Proclossiana eunomia	-8.67	-25.40
Calliphoridae	7.10	-24.98
Muscidae - Coenosiinae	4.60	-24.69
Limoniinae	2.41	-24.34

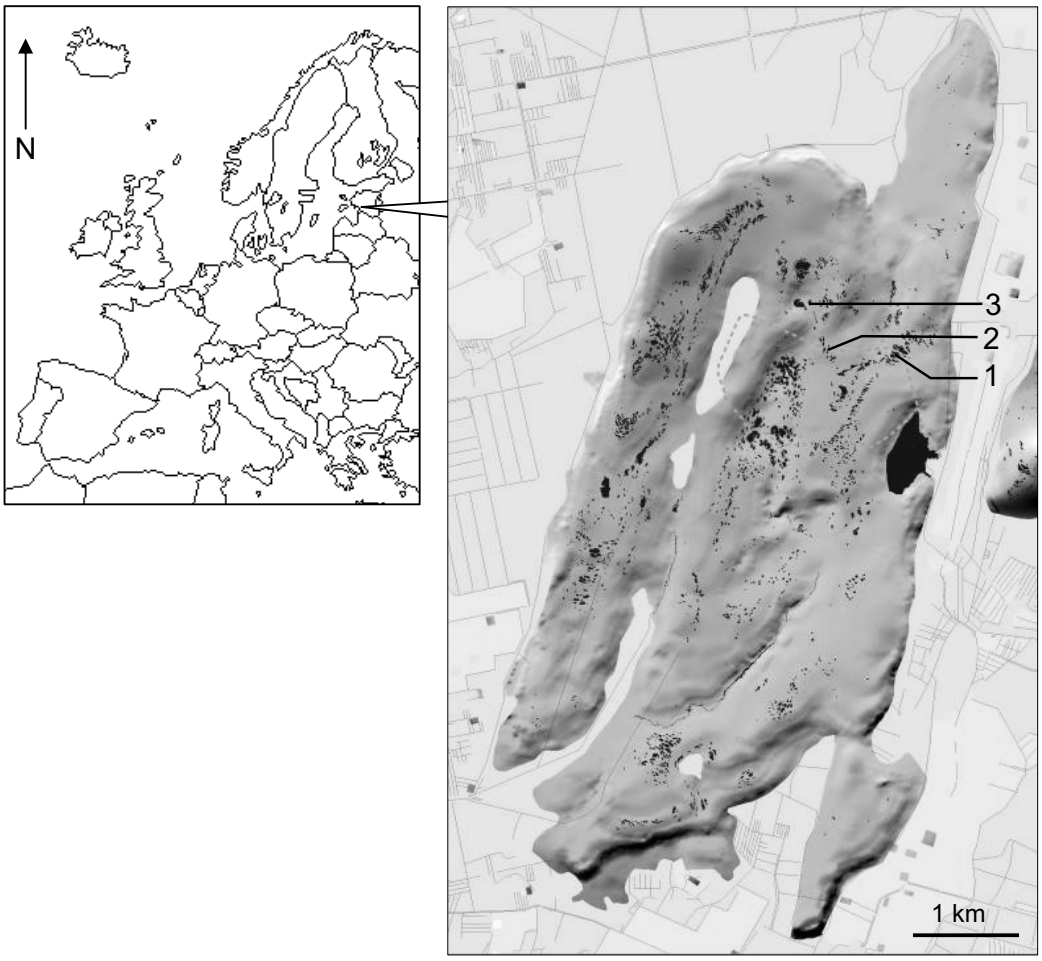
	Omnivores	Carnivores	Herbi-detritivores
SOM	0.10 (0-0.37)	0.09 (0-0.33)	0.14 (0-0.47)
DOC	0.14 (0-0.49)	0.13 (0-0.44)	0.18 (0-0.63)
Sphagnum	0.09 (0-0.33)	0.08 (0-0.29)	0.12 (0-0.42)
Vascular plants	0.11 (0-0.38)	0.10 (0-0.34)	0.14 (0-0.49)
Periphyton	0.49 (0.37-0.60)	0.55 (0.44-0.65)	0.34 (0.18-0.49)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

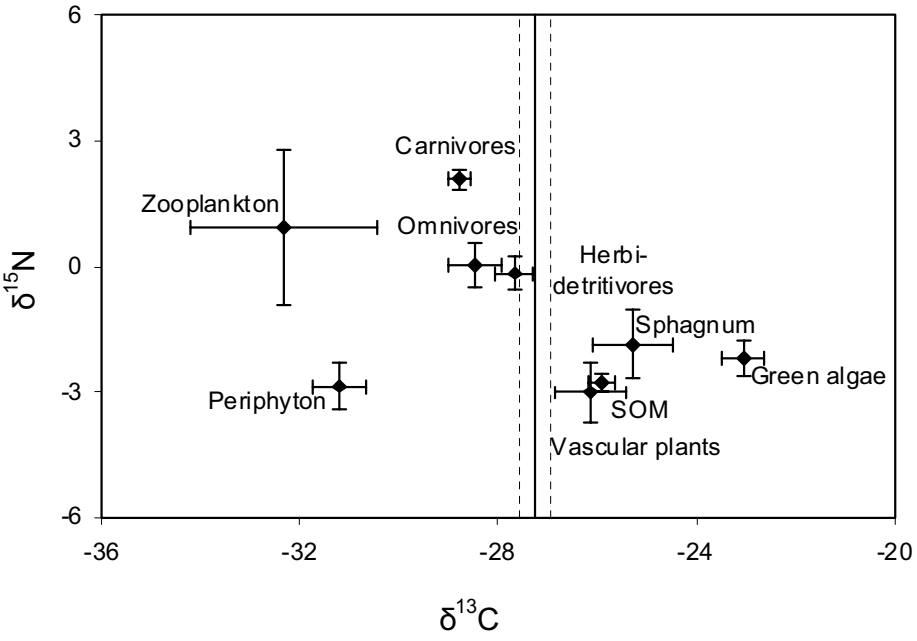
			Zygoptera nymphs		Anisoptera nymphs		Adult Heteroptera			Adult Coleopetera			Midge larvae
PLFA	SOM	Zoo-plankton	*Enallagma cyathigerum*	*Lestes sponsa*	*Leucorrhinia albifrons*	*Aeshna juncea*	*Corixa dentipes*	*Cymatia bonsdorffii*	*Notonecta lutea*	*Ilybius aenescens*	*Ilybius guttiger*	*Laccophilus poecilus*	*Chaoborus* spec.
Methane Oxydizing Bacteria Type 1													
16:1ω8c	0.5		0.0	0.0	0.1	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	0.6	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*	*0.7*	*0.4*	*0.3*	*0.6*	*0.5*	*0.6*
Methane Oxydizing 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	0.3
Total	*2.8*	*0.2*	*0.2*	*0.0*	*0.2*	*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
a15:0	4.1	0.2											
i16:0	1.5	0.2	0.1			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	0.1		0.1	0.2	0.4
br17:0	0.2	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*	*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	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*	*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	7.2	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	20.9	13.2	9.3	12.1	24.0	7.4

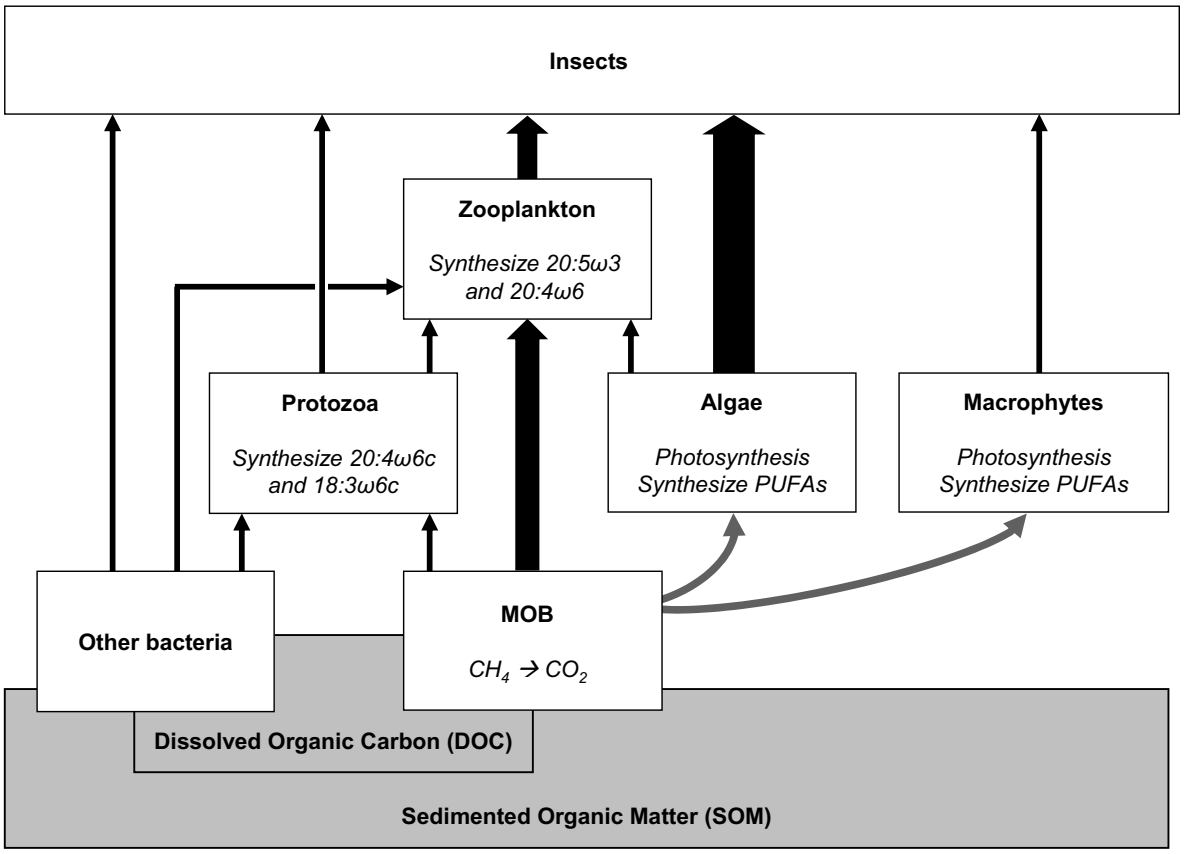
20:5ω3	0.6	3.9	14.3	16.8	13.6	10.9	20.2	16.8	11.3	8.4	9.7	14.1	22.6
Total	*4.5*	*5.2*	*38.8*	*37.3*	*39.5*	*36.3*	*41.4*	*44.9*	*47.4*	*52.6*	*51.2*	*42.2*	*39.9*
Monounsaturated													
16:1ω9t		0.1					0.1						0.2
16:1ω9c	0.6				0.6	0.1		0.3				0.1	
16:1ω7c	4.2	10.0	1.6	1.2	2.0	1.8	3.6	2.8	2.3	1.7	2.4	5.9	2.2
16:1ω6c	0.7		0.2	0.2	0.2	0.3	0.4	0.4	0.1		0.2	0.3	0.3
16:1ω5c	0.6	0.2	0.1		0.1	0.2	0.2				0.1	0.2	0.2
17:1ω6c	0.4	0.3	0.1		0.1	0.2	0.2	0.4	0.3			0.2	0.1
18:1ω11t	0.9	0.3			0.2			0.3					
18:1ω9c	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
18:1ω9t	1.6	0.1			0.2								
18:1ω7c	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
18:1ω5c	0.2				0.1	0.2	0.1						
20:1ω?	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, 18:1ω7c, the branched PLFAs and the PLFAs with cyclopropyl rings. The ? in 20:4ω? means that the position of the double bond nearest to the aliphatic end of the molecule was not identified.

			Zygoptera larvae		Anisoptera larvae		Adult Heteroptera			Adult Coleoptera			Midge larvae
PLFA	SOM	Zoo-plankton	*Enallagma cyathigerum*	*Lestes sponsa*	*Leucorrhinia albifrons*	*Aeshna juncea*	*Corixa dentipes*	*Cymatia bonsdorffii*	*Notonecta lutea*	*Ilybius aenescens*	*Ilybius guttiger*	*Laccophilus poecilus*	*Chaoborus* spec.
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	-33.4	-33.3	-34.1	-34.8	-32.5	-32.0	-32.8	-32.9	-36.9
16:1ω7c	-36.7	-27.9	-31.3	-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	-33.2	-32.1	-33.8	-34.1	-32.3	-30.8	-31.7	-32.4	-38.1
18:1ω11t	-35.5												
18:1ω9c	-32.5	-30.6	-33.3	-34.1	-33.4	-32.5	-34.4	-34.5	-32.5	-31.3	-32.8	-32.8	-36.4
18:1ω9t	-35.6												
18:1ω8c	-38.0												
18:1ω7c	-35.3	-30.6	-33.2	-33.2	-33.5	-31.7	-35.9	-31.6	-33.5	-32.9	-29.8	-33.7	-34.4
18:2ω6c,9c									-32.7	-32.9	-33.1		
18:3ω6			-40.5	-41.4	-37.5	-39.5	-37.2	-42.3	-37.5	-39.2	-40.6	-40.3	-40.9
18:3ω4	-34.2												
cy19:0	-35.6		-32.3				-37.6						
20:4ω?	-45.5	-47.1	-33.6	-39.3	-33.2	-34.2	-36.7	-36.5	-34.0	-34.2	-35.4	-34.5	-40.3
20:5ω3	-30.6	-34.5	-37.1	-37.2	-36.0	-34.0	-32.1	-39.2	-35.3	-34.8	-37.4	-37.2	-36.2


N
3
2
1
1 km


6
3
0
-3
-6
-36
-32
-28
-24
-20
δ15N
δ13C
Carnivores
Zooplankton
Omnivores
Herbi-
detritivores
Sphagnum
Green algae
Periphyton
SOM
Vascular plants


Insects
Zooplankton
Synthesize 20:5ω3 and 20:4ω6
Protozoa
Synthesize 20:4ω6c and 18:3ω6c
Algae
Photosynthesis Synthesize PUFAs
Macrophytes
Photosynthesis Synthesize PUFAs
Other bacteria
MOB
$CH_4 \rightarrow CO_2$
Dissolved Organic Carbon (DOC)
Sedimented Organic Matter (SOM)