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# Linkages between benthic microbial and freshwater insect communities in degraded peatland ditches

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ABSTRACT

Many wetlands are heavily modified and identifying the environmental drivers of indicator groups like aquatic insects is complicated by multiple stressors and co-varying environmental factors. Yet, incorporating data from other biological groups, such as microbial communities, potentially reveals which environmental factors are underpinning insect community composition. In the present study we investigated the application of benthic microbial community composition, as determined by phospholipid fatty acid (PLFA) analysis, alongside aquatic insect data in 25 peatland ditches in the province of North Holland, The Netherlands. We applied clustering and principal component analysis to a matrix of 26 PLFAs to group ditches by the microbial community. Generalized linear models were used to examine correlations between microbial PLFAs, insects, vegetation (emergent and submerged) and abiotic factors. The ratio of heterotrophic (e.g. sulphate reducing bacteria) to autotrophic (e.g. algae and cyanobacteria) derived PLFAs could be estimated as the ratio between saturated and branched to monounsaturated and polyunsaturated fatty acids (SB/MP). SB/MP was correlated with insect community composition, differences in water chemistry (in particular bicarbonate, sulphate and nutrients) and vegetation cover in the ditches. Moreover, ditches distinguished by their microbial communities differed in the number of insects they supported with differences most pronounced for Odonata, Trichoptera and *Chironomus* larvae. This study demonstrates that integrating microbial and aquatic insect community data provides insight into key environmental drivers in modified aquatic ecosystems and may facilitate the development of remediation strategies for degraded wetlands.



## 1. Introduction

Wetlands are some of the most heavily impacted environments in the world. In Europe alone, wetland loss is estimated in excess of 50% of the original land area while in New Zealand it is thought to be as high as 90% (see Moser et al., 1996 and references therein). The productivity of wetland soils combined with a reliable water supply has resulted in the drainage and conversion of wetlands to agricultural land. Many of these wetlands contain large numbers of drainage ditches which potentially support a range of aquatic biota reminiscent of the natural wetland environment. However, determining the ecological conditions of such heavily modified environments is complicated by the presence of multiple stressors and a lack of suitable reference conditions for comparative ecological assessments. Aquatic invertebrates are widely used as indicators of wetland ecosystem status because they reflect conditions relating to nutrients, macro-ions, salinity and habitat structure, among other factors (Lunde and Resh, 2012; O'Toole et al., 2008; Scheffer et al., 1984; Smith et al., 2007; Van der Hammen, 1992; Verdonschot et al., 2012). Within the invertebrate community insects are particularly good indicators of overall ecosystem conditions as the majority of insects have a terrestrial phase in their life-cycle (Crichton et al., 1978; Elliott and Humpesch, 2010; Nilsson, 2005). However, in heavily modified wetlands, relating patterns of insect diversity to abiotic conditions is complicated

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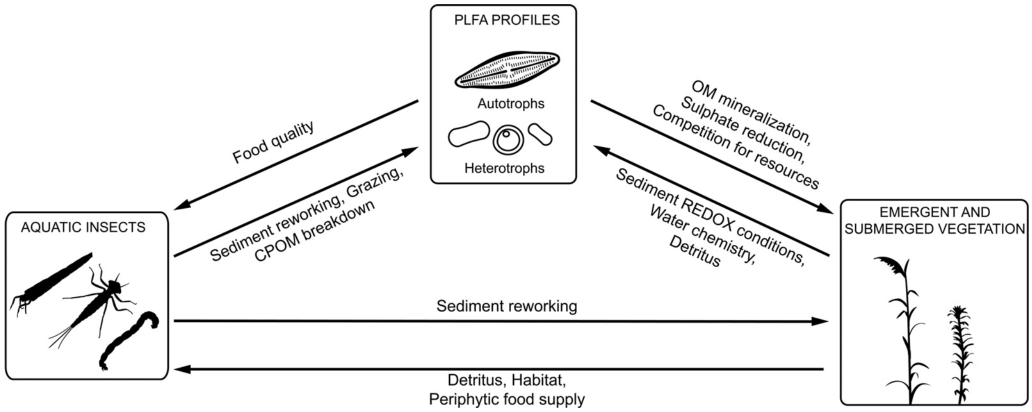


**Fig. 1.** Simplified diagram showing the interconnections between benthic microbial community (bacteria and algae), aquatic insects and aquatic vegetation.

by interaction effects. For example, increased nutrients may supply food (i.e. by stimulating epiphytic algae growth) to insect species, but concomitantly result in stress by causing diurnal fluctuations in oxygen concentrations, thus limiting the distribution of these same species.

Analysis of other groups of organisms, such as microbes, may reveal additional information on environmental factors that are involved in driving shifts in insect diversity. Determination of benthic microbial composition, by phospholipid fatty acid (PLFA) analysis is a well-established in situ measure of microbial biomass and community composition (Kaur et al., 2005; Piotrowska-Seget and Mrozik, 2003). Yet, ecosystem studies linking aquatic insects and microbes are rare outside of food web studies (Goedkoop et al., 1998; Peeters et al., 2004; Vos et al., 2002). Microbial lipids and polyunsaturated fatty acids (PUFAs) in particular, are essential dietary requirements of insects. Sediment dwelling insects in-turn can influence microbial communities through grazing (Goedkoop et al., 1997; Traunspurger et al., 1997), sediment mixing and detrital processing (Hunting et al., 2012). Moreover, aquatic insects in lentic environments depend on vegetation for habitat and microbes (bacteria and algae) influence aquatic macrophytes by mineralizing organic matter and competing for resources (e.g. light and nutrients). Rooted vegetation in-turn influences microbes by altering sediment conditions, water chemistry and detrital composition (see Fig. 1).

Benthic microbial community composition analysed in relation to environmental drivers, such as the degree of vegetation cover, hydrological regime, nutrients, pH and edaphic conditions, can reveal the role of these drivers in underpinning ecosystem status (Bååth et al., 1995; Boon et al., 1996; Gao et al., 2005). Moreover, integration of microbial and insect data can be applied to get a better overview of wetland health, particularly in modified landscapes which lack suitable reference conditions. An advantage of PLFA analysis is that it provides an accurate measure of the living and active microbial community because PLFAs are quickly hydrolyzed in dead cells (Findlay et al., 1989; Harvey et al., 1986; Sundh et al., 1997). Furthermore, PLFAs can be used to identify the presence of different microbial groups because they differ in their fatty acid compositions (Kaur et al., 2005). Comparison of different biological communities, such as benthic microbial communities and aquatic insects provides information on both benthic and littoral communities, giving a wider overview of factors which affect the entire aquatic community.

The drainage ditches of North Holland's peatlands are some of the most degraded wetland environments in Western Europe. These wetlands are remnants of a once vast system of river deltas and raised bogs that covered much of The Netherlands (Van Dam, 2001; Van Eerden et al., 2010). Regular vegetation clearance and sediment dredging is performed to maintain drainage ditches and a strict hydrological regime is applied to ensure stable water tables and increased productivity of the surrounding agricultural land. External inputs of River Rhine waters contribute carbonate and sulphate to the peatlands, which in turn increases peat mineralization and the release of nutrients (e.g. by facilitating the anaerobic mineralization of peat and release of sediment bound phosphates) (Lamers et al., 2002; Smolders et al., 2006). The inlet of these mineral rich waters, in combination with oxidation arising from wetland drainage and the diffuse release of nutrients from the surrounding agricultural land, are underlying the eutrophication of North Holland's peatlands (Lamers et al., 2002; Sinke et al., 1990).

In the present study we assess if microbial data can reveal habitat suitability for aquatic insects and help to identify key environmental drivers of ditch communities in North Holland's peatlands. We used monitoring data (collected by the local Water Authority as part of standard monitoring) available for insects, water chemistry and submerged and emergent vegetation. In addition we collected sediment samples for microbial PLFA analysis and measurements to estimate emergent vegetation cover. Using clustering and principal component analysis (PCA) we classified 25 peatland ditches, based on microbial community composition, and investigated how this classification related to insect communities, submerged and emergent vegetation and abiotic conditions in peatland ditches. We then employed generalized linear models (GLMs) and canonical correspondence analysis (CCA) to determine the variation in insect community composition explained by microbial and environmental (abiotic and vegetation) predictor variables. We expect differences in vegetation and abiotic conditions between ditches to be reflected by microbial community composition and anticipate that microbial PLFAs will provide insight into factors underlying aquatic insect community composition in North Holland's peatlands.

## 2. Methods

### 2.1. Study sites and environmental data collection

Monitoring locations were sampled for macroinvertebrates, aquatic vegetation and abiotic variables in 2011 as part of the annual monitoring of surface waters undertaking by the North Holland Water Authority, Hoogheemraadschap Holland's Noorderkwartier (HHNK). Based on the dimensions of the water body, soil type and salinity 25 locations were selected from a larger dataset to obtain a set of samples from similar habitats. The locations were all ditches, situated in peat areas with average chloride levels less than 1000 mg $Cl^{-}$ $L^{-1}$ and representing a range in water quality (e.g. nutrients, sulphate and bicarbonate), from