



## Changes in plant community structure and soil biota along soil nitrate gradients in two deciduous forests



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### ABSTRACT

Anthropogenic nitrogen (N) deposition is a serious threat to biodiversity and the functioning of many ecosystems, particularly so in N-limited systems, such as many forests. Here we evaluate the associations between soil nitrate and changes in plant community structure and soil biota along nitrate gradients from croplands into closed forests. Specifically, we studied the composition of the understory plant and earthworm communities as well as soil microbial properties in two deciduous forests (Echinger Lohe (EL) and Wippenhauser Forst (WF)) near Munich, Germany, which directly border on fertilized agricultural fields. Environmental variables, like photosynthetically active radiation, distance to the edge and soil pH were also determined and used as co-variables. In both forests we found a decrease in understory plant coverage with increasing soil nitrate concentrations. Moreover, earthworm biomass increased with soil nitrate concentration, but this increase was more pronounced in EL than in WF. Soil microbial growth after addition of a nitrogen source increased significantly with soil nitrate concentrations in WF, indicating changes in the composition of the soil microbial community, although there was no significant effect in EL. In addition, we found changes in earthworm community composition along the soil nitrate gradient in WF. Taken together, the composition and functioning of forest soil communities and understory plant cover changed significantly along soil nitrate gradients leading away from fertilized agricultural fields. Inconsistent patterns between the two forests however suggest that predicting the consequences of N deposition may be complicated due to context-dependent responses of soil organisms.

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### Introduction

Forest edges have received much attention in ecology and ecosystem management, since they represent a transition zone between the forest habitat and adjacent ecosystems. Surrounding areas such as pasture and cropland can trigger changes in the species composition and nutrient cycles across edges (Murcia 1995; Ries et al. 2004; Harper et al. 2005). Additionally, differences in microclimate occur between the two sides of the edge, which can also lead to changes in the rate of decomposition and nutrient mobilization (Murcia 1995). Edges are also subjected to the influx of chemical compounds from the atmosphere or via drift from surrounding land (Thimonier et al. 1992; Wuyts et al. 2008). Therefore, increased deposition of potentially acidifying and eutrophying ammonium, nitrate and sulphate depositions at the edge has been reported (Wuyts et al. 2008). Such influences result in differences in

top soil properties and understory plant species composition with increasing distance to the edge (Matlack 1994; Wuyts et al. 2011).

Nitrogen (N) is one of the key elements affecting the diversity, dynamics and functioning of terrestrial, freshwater and marine ecosystems. Many organisms have adapted to low levels of N (Vitousek et al. 1997); moreover, the availability of N strongly influences the growth and abundance of organisms (Vitousek and Howarth 1991). Anthropogenic N deposition sources, like agriculture or combustion of fossil fuels, thus exert strong effects on the biodiversity and functioning of many ecosystems (Sala et al. 2000). In N-limited ecosystems, such as forests, changes in herbaceous layer biodiversity and composition are of special interest due to their functional relevance and fast response to changes in N availability (Tamm 1991; Gilliam 2006; Gilliam 2007; Bernhardt-Römermann et al. 2010).

Elevated N availability has often been reported to increase plant productivity, but to decrease plant diversity by favoring the few species that are most efficient in N uptake (Gilliam 2006; Harpole and Tilman 2007; Clark and Tilman 2008). For instance, comprehensive long-term studies within the BioCON experiment

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(Reich et al. 2001) showed an increase in plant productivity in response to N addition, but a decrease in soil microbial biomass (Dijkstra et al. 2005; Eisenhauer et al. 2012), plant (Reich 2009) and soil animal diversity (Eisenhauer et al. 2012). In contrast to the above-mentioned studies, Bernhardt-Römermann et al. (2010) found increasing functional diversity of plants with increasing soil N levels in the deciduous forest investigated in the present paper. However, as typical for studies in terrestrial ecology, belowground responses to global change agents, such as fertilization effects, are still less well explored. Moreover, it is unclear if fertilization of agricultural fields influences the composition and functioning of adjacent ecosystems.

Soil organisms play major roles in several ecosystem functions; for example, promoting plant productivity, regulating nutrient mineralization and promoting decomposition of organic matter (Neher 1999; Wardle et al. 2004). Thus, shifts in soil food web composition in response to fertilization may cause pronounced alterations in ecosystem functioning. Given that different groups of soil microorganisms use different resources with respect to complexity and quality (Griffiths et al. 1999; Kramer and Gleixner 2006), fertilization may cause compositional shifts in communities of soil microbes and animals (Frey et al. 2004).

Although previous studies showed inconsistent responses of soil organisms to N inputs (e.g., Niklaus and Körner 1996; Zak et al. 2000; Dijkstra et al. 2005), some general patterns tend to emerge. For instance, based on a meta-analysis, Treseder (2008) hypothesized that N input through fertilization negatively affects soil microbial biomass due to one or more mechanisms including alterations in soil pH, carbon and N availability, and below- and aboveground productivity of plants. Further, N input may decrease rhizodeposition fueling rhizosphere communities (Högberg et al. 2010; Eisenhauer et al. 2012). Treseder (2008) concluded that N addition has overall negative effects on microbial biomass and that long fertilization periods and large amounts of N even intensify the reduction of microbial biomass (Treseder 2008). Likewise, DeForest et al. (2004) reported decreased microbial enzyme activity after N fertilization, indicating shifts in the functioning of soil microbial communities. Moreover, root colonization and species richness of mycorrhizal fungi have been reported to decrease due to higher N availability (Egerton-Warburton and Allen 2000; Lilleskov et al. 2002a,b).

Responses of different groups of soil organisms to fertilization may however differ. Several experiments have shown that both organic and inorganic N fertilizers can have beneficial effects on earthworm populations (Edwards and Lofty 1982; Timmerman et al. 2006). For example, higher nutrient availability through organic N has been shown to increase earthworm biomass and numbers (Edwards and Lofty 1982; Hansen and Engelstad 1999), although extremely high levels of N input may inhibit their proliferation (Haynes and Naidu 1998). The effects of inorganic N fertilizers can be explained by an increasing amount of plant material and the subsequent higher amount of decomposing organic matter (Edwards and Lofty 1982). Plant litter decomposition has long been recognized as an essential process for organic matter turnover and nutrient fluxes in most ecosystems. The subsequent release of carbon and nutrients represents the primary source of nutrients for plants and microbes (Berg and McClaugherty 2008). Rates of plant litter decomposition and nutrient mineralization are, in turn, influenced by litter quality; higher nitrogen contents mostly enhance decomposition.

Despite all this previous work, there is still a lack of understanding of the responses of ecosystems to N addition effects (West et al. 2006; Bardgett and Wardle 2010; Decaëns 2010). Most studies on high soil N concentrations are either based on permanent plot observations or on studies along environmental gradients

(Brunet et al. 1998). The present study focuses exclusively on soil nitrate gradients in forest ecosystems. We investigated the associations between soil nitrate concentrations and plant community properties, soil biota and functioning in forests that are adjacent to fertilized agricultural fields. To achieve this, we studied understory plant community composition, earthworm communities and soil microbial properties in two deciduous forests near Munich, Germany. We used an observational approach to investigate N gradients as was also done by Bernhardt-Römermann et al. (2007, 2010) in one of the forests investigated in the present study. Such gradients in soil nitrate concentrations could arise from the drift and/or lateral flow of mineral N applied via fertilizers. Given inconsistent results in previous studies, we investigated two forests differing in tree, understory plant and soil community composition. We expected soil nitrate gradients leading away from fertilized agricultural fields to be associated with significant changes in plant community composition and cover as well as in the diversity of soil organisms of adjacent forest stands (Bernhardt-Römermann et al. 2007). More specifically, we hypothesized an increase in diversity and productivity of plant communities (Bernhardt-Römermann et al. 2010), a decrease in soil microbial biomass (Treseder 2008) and an increase in earthworm biomass (Edwards and Lofty 1982) with increasing nitrate concentrations accompanied by significant alterations in soil processes.

## Materials and methods

### Study sites and sampling design

We used two study sites located in two different deciduous forests close to the city of Munich, Germany. The first one is the 'Echinger Lohe' (EL), a nature reserve located 20 km north-east of Munich on the 'Münchner Schotterebene', a plain which was formed at the end of the last ice age. The texture of this Leptosol changes from carbonate-rich sandy soil toward humus-rich sandy – loamy gravel with increasing distance to the edge. The forest patch covers an area of about 24 ha and is surrounded by intensively used agricultural land. The dominating tree species are *Carpinus betulus* L., *Quercus robur* L. and *Acer pseudoplatanus* L., and the understory vegetation is dominated by *Colchicum autumnale* L., *Anemone nemorosa* L. and *Carex alba* Scop. The surrounding fields represent a diverse mixture of conventionally fertilized barley, rye and potato fields.

The second forest site is the 'Wippenhauser Forst' (WF), which is located north of Freising, approximately 40 km northeast of Munich. The texture of this Cambisol is dominated by loess loam and sandy gravel belonging to the upper fresh water molasse (Tertiary). Similar to the EL, the WF has a sharp border between the forest and agricultural field at its southern edge. In 2012 rye was grown on the conventionally fertilized agricultural field. The forest overstory is dominated by *Fagus sylvatica* L. and *A. pseudoplatanus* L., while the understory is dominated by *Carex brizoides* L. and *Rubus fruticosus* L.

Twenty monitoring plots were set up per forest, and all measurements were done in both forests in May 2012. In EL, one transect was set up with 20 plots (1 m × 1 m) spaced at 20 m intervals along a soil nitrate gradient (Fig. S1) reported in a previous study (Bernhardt-Römermann et al. 2007). In WF, two transects (Fig. S2, 10 plots each, 1 m × 1 m, spaced at 20 m intervals) were set up leading from the edge to the center of the forest.

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