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Bacterial and fungal response to nitrogen fertilization in three coniferous forest soils

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Abstract

Forest soil carbon (C) pools may act as sinks for, or sources of, atmospheric carbon dioxide, while nitrogen (N) fertilization may affect the net exchange of C in forest ecosystems. Since all major C and N processes in soil are driven by soil microorganisms, we evaluated the effects of N fertilization on biomass and bacterial and fungal activity in soils from three Norway spruce forests with different climatic and N availability conditions. N deposition and net N mineralization were higher at the sites in southern Sweden than at the site in northern Sweden. We also studied the extent to which N fertilization altered the nutrient(s) limiting bacterial growth in soil. We found that on average microbial biomass was reduced by ~40% and microbial activity by ~30% in fertilized plots. Bacterial growth rates were more negatively affected by fertilization than fungal growth rates, while fungal biomass (estimated using the phospholipid fatty acid (PLFA) 18:2ω6,9) decreased more than bacterial biomass as a consequence of fertilization. The microbial community structure (indicated by the PLFA pattern) was changed by fertilization, but not in the same way at the three sites. Soil bacteria were limited by a lack of carbon in all forests, with the carbon limitation becoming more evident in fertilized plots, especially in the forests that had previously been the most N-limited ones. This study thus showed that the effects of N fertilization differed depending on the conditions at the site prior to fertilization.

Keywords: Fertilization; Nitrogen; Coniferous forest soil; Bacteria; Fungi; PLFA; Limiting nutrient

1. Introduction

Aboveground production in boreal and temperate forests is most often limited by the availability of nitrogen (N) (Tamm, 1991). Fertilization with N has therefore been widely practiced, especially in the most N-limited forest ecosystems, e.g. in northern Sweden. Typically, a single dose of 150 kg N ha^{-1}, 10 years before harvest, increases stem production by 10–20 m^3. Forest fertilization decreased from a peak in the late 1980s, when 200,000 ha of Swedish forests were annually fertilized, to the present level of ~30,000 ha (Högbom and Jacobson, 2002). Intensive forest management with optimized nutrient fertilization is, however, now being discussed as a means of increasing biofuel production in forests with low natural conservation value, in order to reduce society's dependence on fossil fuels. During the 1980s the direct effects of forest fertilization were of interest, but during the past decade the fertilization of forests has been used to simulate and study the effects of elevated input of N by deposition, which is one of the problems associated with global change (Wright and Rasmussen, 1998; Aber and Magill, 2004). Apart from increasing tree growth, fertilization of forests with N affects the ecosystem in other ways, such as reduced biodiversity of plants, mosses and lichens (Olsson and Kellner, 2006, and references therein), ectomycorrhizal fungi (Fransson et al., 2000), and soil micro- and mesofauna (Lindberg and Persson, 2004). Other serious effects include nitrate leaching, leading to eutrophication and health risks, and denitrification and emission of N_2O, which is a potent greenhouse gas.

Soil microbial biomass and activity have frequently been reported to decrease after N fertilization of forests

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(e.g. Bååth et al., 1981; Arnebrant et al., 1996; Thirukkumaran and Parkinson, 2000; Sjöberg et al., 2003; Bowden et al., 2004; Knorr et al., 2005). In addition, the accumulation of organic matter and an increase in soil C storage in fertilized forest soils have been observed after N fertilization (Nohrstedt et al., 1989; Mäkipää, 1995; Canary et al., 2000; Adams et al., 2005; Olsson et al., 2005). Less is known about the effects on specific groups of microorganisms. Ectomycorrhizal fungi constitute an important group of soil microbes that are sensitive to elevated N levels (Wallenda and Kottke, 1998). Nitrogen fertilization often leads to changes in the ectomycorrhizal fungal community on the root tips of the trees (Kårén and Nylund, 1997; Fransson et al., 2000) and production of fruiting bodies (Wiklund et al., 1995), mycorrhizal roots (Berch et al., 2006) and external mycelia (Nilsson and Wallander, 2003). The microfungal species composition has also been seen to be altered following N fertilization with ammonium nitrate or urea (Arnebrant et al., 1990) and different DNA profiles of the 16S rDNA gene for *Nitrobacter* have been found in plots subjected to different N treatment (Compton et al., 2004).

Others have used indirect methods to reveal changes in microbial community composition after fertilization, such as phospholipid fatty acid (PLFA) patterns (Leckie et al., 2004; Waldrop et al., 2004; Grønli et al., 2005; Högberg, 2006; Högberg et al., 2007) or community-level physiological profiles (Compton et al., 2004; Leckie et al., 2004). Few have attempted to separate the effects on fungi and bacteria, and then only biomass- and not growth-related variables were studied (Söderström et al., 1983; Compton et al., 2004; Frey et al., 2004; Högberg, 2006; Wallenstein et al., 2006). Tietema (1998) suggested that the soil microbial community changes depending on the soil nitrogen status, becoming more fungi than bacteria dominated at N-limited sites.

The effects of N fertilization on the soil microbial community have thus been relatively little studied. In this study we examined the response of soil microorganisms to long-term (over 10 years) nitrogen fertilization in three coniferous forest soils with different climatic and nutrient conditions. Emphasis was placed on differentiating the effects on fungal and bacterial biomass and activity (growth). These specific measures were combined with conventional biomass and activity measurements. A recently developed method of determining nutrients limiting bacterial growth (Aldén et al., 2001) was also applied. We expected bacteria to become more carbon limited in fertilized soils and at sites with high nutrient deposition.

2. Materials and methods

2.1. Site descriptions

Soil samples were collected at three Norway spruce forest sites in Sweden with different climatic and nutrient availability conditions. Flakaliden is a boreal, highly N-limited site, in northern Sweden (64°07′ N, 19°27′ E), whereas Asa in south-eastern (57°08′ N, 14°45′ E, altitude 240 m) and Skogaby in south-western Sweden (56°33′ N; 13°13′ E; altitude 95–110 m) represent temperate forests in a milder climate. Nutrient availability is increased by, for example, higher deposition of N and other nutrients at the southern sites. The atmospheric deposition of inorganic N increases from ~3 kg ha^{-1} at the northern site to ~10 at the south-eastern, and to more than 20 kg ha^{-1} at the south-western site. N mineralization rates are higher in the south, which may be due to the higher mean annual temperatures: 7.1 °C at the south-western (SW) site (Skogaby) and 5.2 °C at the south-eastern (SE) site (Asa), compared with 1.2 °C at the northern (N) site (Flakaliden). Precipitation is higher in the south, with a mean annual precipitation of about 1100 mm at the SW site and ~700 mm at the SE site; although with frequent early summer droughts at both these sites. At the N site the mean annual precipitation is approximately 600 mm, of which more than one-third falls as snow, and snow usually covers the frozen ground from mid-October to early May. Tree productivity is higher at the southern sites (~10 m^3 ha^{-1} yr^{-1} at the SE and SW sites) than at the N site (3.2 m^3 ha^{-1} yr^{-1}).

All three sites are planted with Norway spruce (*Picea abies* (L.) Karst.); at the SW site in 1966 as a second-generation spruce forest on a former *Calluna* heath land, at the SE site in 1975 and at the N site in 1963, the latter two on historically long-term forest sites after prescribed burning and soil scarification. The soils at all three sites are classified as Haplic Podzols; at the SW site on loamy-sand till, at the SE site on sandy till, while the soil at the N site is a well-developed iron podzol, on silty-sandy till. The average depth of the O layer was 7 cm at the SW site, 6 cm at the SE site and ~3 cm at the N site. Field vegetation is sparse at the test sites in the SW and the SE sites (the latter with patches of moss), while the understory vegetation at the N site is dominated by *Vaccinium vitis–idaea*, *Vaccinium myrtillus*, *Deschampsia flexuosa* and *Empetrum* spp.

The treatment applied to all sites was similar with four replicate blocks (approx. 100–500 m apart). The treatments applied were: control (no treatment), fertilization, fertilization + irrigation and irrigation alone. Nitrogen fertilizing agents have been added at the SW site since 1988 as solid ammonium sulphate, at an annual rate of 100 kg N ha^{-1}. In the fertilization + irrigation treatment less nitrogen was applied in 1994 and since (mean application rate 35 kg N ha^{-1}). The SE and the N sites have been fertilized since 1987, with 50–100 kg N ha^{-1} annually, in order to optimize timber production. The fertilizers were applied as a complete nutrient addition (also including K, P, Mg, K, Ca, S, Fe, B, Mn, Cu and Zn), based on previous year foliar nutrient status, nutrient concentrations in the soil water and predicted growth response. The mixture was added as solid salts at the beginning of June in fertilized plots, or injected into the irrigation water and supplied daily during the growing season (June–mid-August) in