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SHORT COMMUNICATION

# Changes in microbial community structure in soil as a result of different amounts of nitrogen fertilization

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**Abstract** The changes in size, activity and structure of soil microbial community caused by N fertilization were studied in a laboratory incubation experiment. The rates of N fertiliser applied ($KNO_3$) were 0 (control), 100 and 2,000 μg N $g^{-1}$ soil. Despite no extra C sources added, a high percentage of N was immobilized. Whereas no significant increase of microbial C was revealed during incubation period, microbial growth kinetics as determined by the substrate-induced growth-response method demonstrated a significant decrease in the specific growth rate of microbial community in soil treated with 2,000 μg N $g^{-1}$ soil. Additionally, a shift in microbial community structure resulting in an increase in fungal biomarkers, mainly in the treatment with 2,000 μg N $g^{-1}$ soil was visible.

**Keywords** Nitrogen immobilization · Microbial community structure · Microbial biomass

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## Introduction

The immobilization of nitrogen by soil microorganisms is of central importance for sustaining a high soil quality. It protects nitrogen from being lost by leaching and volatilisation and stimulates a prolonged positive effect for plant nutrition due to re-mineralization of the initially immobilized nitrogen over time.

Interestingly, it has been shown in a number of studies that nitrogen immobilization can be independent from the amount of dissolved organic carbon in soil (Jackson et al. 1989; Recous et al. 1990; Hadas et al. 1992; Yevdokimov and Blagodatsky 1993). Consequently, moderate loads of nitrogen (≤200 μg N $g^{-1}$) can lead to C deficiency or C starvation in soil if adequate amounts of carbon sources are missing, resulting in low C/N ratio in the microbial biomass. Extreme high loads of nitrogen, however, may result in osmotic stress (Kieft et al. 1987), leading to cell death of highly sensitive microbes. Therefore, we postulate that different loads of nitrogen under carbon deficiency change microbial community structure. In our experiment, we used phospholipids as a biomarker for microbial community structure as well as the stress status of a microbial biomass. Due to its high mobility, nitrate is considered as the most critical form of nitrogen in soil both for a sustainable plant growth and for an environmental friendly agriculture; therefore, nitrate was chosen as a model compound in this study.

## Materials and methods

Freshly sampled soil (eutric cambisol, 1.6±0.1% C, 0.14±0.01% N, $pH_{KCl}$ 5.9±0.2; Scheyern, Bavaria, Germany) was sieved through 2 mm and then stored for 2 weeks at 4°C. For the analysis of phospholipids, the soil samples (50 g of dry weight) were placed into 500-ml bottles and amended soil with $KNO_3$ solution. The amounts of nitrate added were 0, 100 and 2,000 μg N $g^{-1}$. Finally, 0.1 M KCl solution was used to adjust to 70% of water holding capacity (WHC); soil samples were incubated at 22°C. Soil samples were taken 30 days after the addition of the different amounts of nitrate. All experiments were done as four independent replicates.

Microbial biomass *C* ($C_{mic}$) in soil was determined by the fumigation–extraction (FE; Vance et al. 1987) and substrate-induced respiration (SIR; Kaiser et al. 1992). Specific growth rates of soil microflora were determined by the substrate-induced growth response method (Panikov and Sizova 1996), which involves simulation of lag and exponential phases of microbial growth after addition of excess quantities of readily decomposable C substrate. Gas samplings were determined hourly during 27 h after glucose addition.

Phospholipids fatty acid (PLFA) analysis was carried out as described by Zelles and Bai (1993) and Gattinger et al. (2002). Individual PLFA markers were used to quantify the relative abundances of specific microbial groups. Bacteria were identified by saturated fatty acids (SATFAs): i15:0, a15:0, n15:0, i16:0, i17:0, a17:0, n17:0, cy17:0, cy19:0 and the monounsaturated fatty acids (MUFAs): 16:1ω7, 16:1 ω9 and 18:1 ω7. From this selection, i15:0, a15:0, i17:0 and a17:0 are specific for Gram-positive bacteria, and cy17:0 and cy19:0 for Gram-negative bacteria (Wiemken et al. 2001; Zelles 1999). Other microbial groups identified with PLFA biomarkers include fungi (18:2ω6; Frostegård and Bååth 1996), microeucaryotes (20:2ω6,9c, 20:3ω6,9,12c, 20:4ω6,9,12,15c; Zelles 1999), actinomycetes (10-methylbranched SATFAs; Zelles 1999) and anaerobic prokaryotes (e.g. methanogenic archaea and *Clostridia* spp. by non-ester-linked PLFAs; Gattinger et al. 2002). Fungi-to-bacteria ratio (Blagodatskaya et al. 2006),

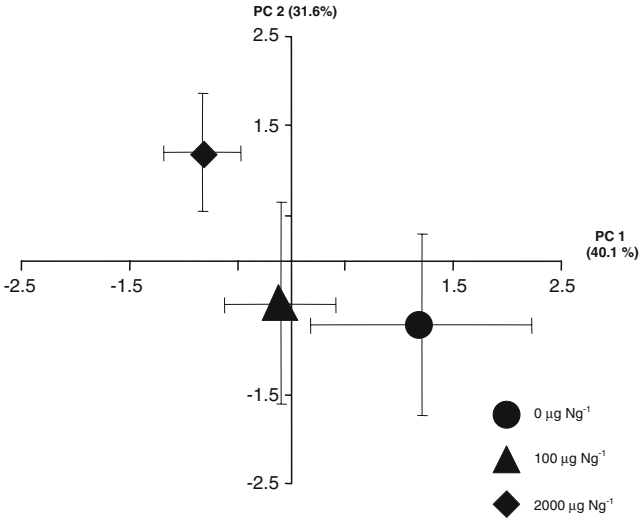


**Fig. 2** Principal component analysis plots of microbial communities in soil samples as determined by PLFA fingerprinting. A *symbol* represents median of four replicates. *Bars* indicate $CL_{0.95}$

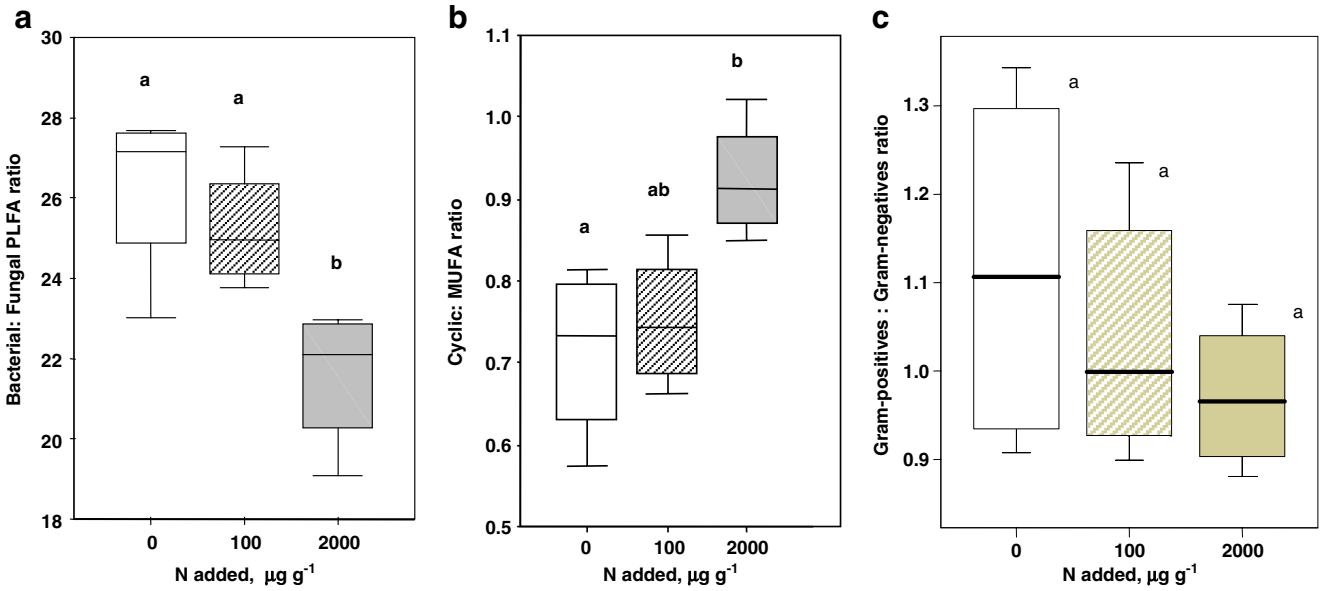


**Fig. 1** Ratio of bacteria to fungi (**a**), cyclopropyl PLFA to monoenoic precursor (**b**), and Gram-positive to Gram-negative bacteria (**c**) in soil 30 days after amendment with $KNO_3$ (0, 100 and 2,000 μg N $g^{-1}$). *Bars* indicate "highest non-outlinear value" and "lowest non-outlinear value" ($n$=4). Different letters (a and b) indicate significance at the $p$=0.05

as well as Gram-negative-to-Gram-positive ratio (Fierer et al. 2003) was used as an indicator for shifts in microbial community structure. The ratio of cyclopropyl PLFA to monoenoic precursor was used to determine the stress status of the microbial biomass (Iyyemperumal and Shi 2007).

## Results and discussion

Although high amounts of the nitrate were immobilized during 30 days in the treatment where 2,000 μg N $g^{-1}$ have been added (data not shown), no significant increase of microbial biomass after nitrate addition was revealed. In all soil treatments, microbial C determined by FE and SIR methods were in the range of 300–400 μg C $g^{-1}$. Total PLFA values also indicated no significant influence of the different treatments on the size of the microbial biomass. In contrast to the unchanged size of microbial biomass, changes in microbial activity occurred. The application of 2,000 μg N–$NO_3$ $g^{-1}$ decreased $\mu_{max}$ approximately to half of that in treatments 0 and 100 (from 0.33–0.35 to 0.17). This might be explained by osmotic stress conditions and C substrate deficiency caused by the extreme amounts of applied nitrogen. This hypothesis is confirmed by our results on PLFA profiling: the cyclic PLFA/MUFA ratio, which is an indicator for general, microbial stress response, was higher in treatment 2,000 (0.93) than that in the control (0.74) and treatment 100 (0.75), thus indicating stress-depending modifications of the PLFA composition of the cell membrane (Bossio and Scow 1998; Guillot et al. 2000; Fouchard et al. 2005).

High percentage of nitrate immobilized has been reported in several other studies. The values range from 34% to up to 70% of the nitrate added (Bristow et al. 1987; Berntson and Aber 2000; Yevdokimov et al. 2005). The mechanisms of the fast nitrate immobilization in soil are still unknown. Colman et al. (2007) hypothesized biological immobilization "following assimilation pathways that we do not understand". It can be supposed that these biological pathways lead to the formation of organic substances with low C/N ratio in microbial biomass, e.g. urea, which has been described for a number of fungi species (Becker-Ritt et al. 2007).

Indeed, in our experiments, we revealed that relative abundance of the PLFA 18:2ω6.9, a biomarker of fungi, increased in the following order: control 0 < treatment 100 < treatment 2,000. Consequently, the ratio between the bacterial and fungal PLFA markers in treatment 2,000 was significantly lower (22.4) than that in control soil and treatment 100 (27.9 and 26.8, respectively; Fig. 1). Obviously Gram-positive bacteria were mainly affected by highest given nitrate concentration. As Gram-negative bacteria were not influenced by the fertilization treatments, the ratio between Gram-positive bacteria and Gram-negative bacteria decreased in the order 0>100>2,000 from 1.03 to 0.90 (Fig. 1). However, the differences were statistically insignificant and represented a trend only. However, overall, a clear shift in PLFA composition in response to the different amendments was visible (Fig. 2). Thus, our data demonstrate that soil is capable for the retention of high amounts of nitrate with unchanged size of microbial biomass but with drastic changes in the structure (increase of fungal biomass) and activity (decrease in specific growth rate) of soil microbial community.

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