Effect of N fertilization on the biomass of soil fungal groups in production grasslands

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Abstract

Nutrient fertilizer application is common in production grasslands as it boosts primary production. We established two 5-year field experiments in the south of Sweden (Lanna, Alnarp). These sites included a two-factorial experiment, i.e. four plant mixtures and two or three levels of nitrogen (N) fertilizer application. The phospholipid- and neutral lipid fatty acid (PLFA, NLFA) analysis was used to estimate the biomass of fungal groups (saprotrophic and arbuscular mycorrhizal fungi, SF and AMF, respectively). At Alnarp, N application was associated with a significant decrease of the AMF biomass, but no trends were seen at Lanna. Similarly, the ratio of AMF/SF decreased with fertilization at Alnarp but not at Lanna. Only one of the plant mixtures (the grass-legume mixture) had an effect in the unfertilized plots, decreasing AMF at Alnarp. Our findings suggest that the impact of N fertilization on soil fungal biomass, specifically the reduction in the abundance of AMF, is site dependent with this group being shown to be more sensitive than SF to N fertilizer application.

Keywords: soil microbial biomass, ley production, diversification, inorganic fertilization

Introduction

Nutrient fertilizer application is generally expected to lead to priming, i.e. a short-term response of microorganisms to easily available nutrients, with the subsequent immediate immobilization and microbial growth before the nutrients eventually become mineralized into plant available forms. This response is thought to more likely involve saprotrophic microorganisms than obligate symbionts, such as arbuscular mycorrhizal fungi. The abundance of AMF has been reported to decrease with N fertilization (Jach-Smith and Jackson, 2018), but contradictory results are also reported, where N fertilization sometimes is found to influence just the AMF community structure (Chen *et al.*, 2014). AMF are generally not selected for by the plant host when nutrients are in excess (Hammer *et al.*, 2011), as is the case under management with mineral fertilization. Plant species diversity enriches AMF functional diversity (Guzmán *et al.*, 2020), but the impact on saprotrophic fungi (SF) is variable. Here we report on the impact of different levels of both plant diversity and mineral N fertilizer application on the soil fungal community of production grasslands. Specifically, we hypothesized that AMF are: (1) more abundant in unfertilized compared to fertilized ley production plots; and (2) promoted by increasing plant species diversity.

Materials and methods

Two field experiments (Alnarp and Lanna) were established in 2013 in the south of Sweden, which has a temperate climate (annual average: 10 °C and 660 mm precipitation). Both sites included a two-factorial experiment, i.e. four plant species mixtures (Table 1) and two N fertilization levels (0 and 60 kg ha^{-1} yr^{-1}), with an additional level (120 kg ha^{-1} yr^{-1}) at Alnarp. Both experiments were replicated in four blocks with a complete randomized factorial design. In the summer of 2018, each experimental plot

was divided into four subplots for taking four soil cores (ø 2.5 cm) to a depth of 20 cm and four 0.25 m^2 squares for estimated plant biomass and to make a plant community inventory. The plant biomass and soil sub-samples were homogenized per plot, and soil samples sieved (2 mm) before all were stored at -20 °C. Plant and soil total N, P and C content and soil texture were analysed. Phospho- and neutral lipid fatty acids (PLFAs and NLFAs) were extracted from the soil samples using the protocol presented by Frostegård *et al.* (1993) and quantified by gas chromatography with a flame ionization detector, and both SF and AMF biomass were estimated. Treatment impact on the microbial biomarkers was analysed with an ANOVA with Tukey's post hoc test. Correlations were detected using parametric Pearson correlation test (P<0.05).

Results and discussion

Both experimental sites had a sandy soil texture: loamy sand at Alnarp and sandy loam at Lanna. The total soil C, N and P were not affected by the applied treatments, but Alnarp showed higher values of all the parameters, especially P.

At Lanna there was a greater amount of AMF biomass than at Alnarp, but no effect of fertilization was detected at the former, while mineral-N fertilizer application significantly decreased the AMF biomass iat Alnarp (9.4, 4.4 and 1.3 nmol g^{-1} for 0, 60 and 120 kg N, respectively) (Figure 1A, B). At this site, the AMF biomass was negatively correlated with the total soil N content (R=-0.29, P<0.05). AMF stimulation under N deficiency in P-rich soils, have been described (Blanke *et al.*, 2005). The total plant N content (%) was positively correlated with the AMF biomass at Alnarp (R=0.48, P<0.001), possibly because the AMF can favour N uptake. The SF biomass was similar at both sites, and no impact of N fertilization was detected (Figure 1C, D). The total plant P content was positively correlated with the

Table 1. Plant species mixtures sown at both experimental sites.

PM1	*Dactylis glomerata* (100%)
PM2	*Phalaris arundinacea* (33%), *Festuca arundinacea* (33%), *D. glomerata* (33%)
PM3	*Medicago sativa* (12.5%), *Trifolium hybridum* (12.5%), *Trifolium repens* (12.5%), *Galega orientalis* (12.5%), PM2 (50%)
PM4	Commercial diverse meadow seed mixture (from 'Pratensis') (75%), PM3 (25%)

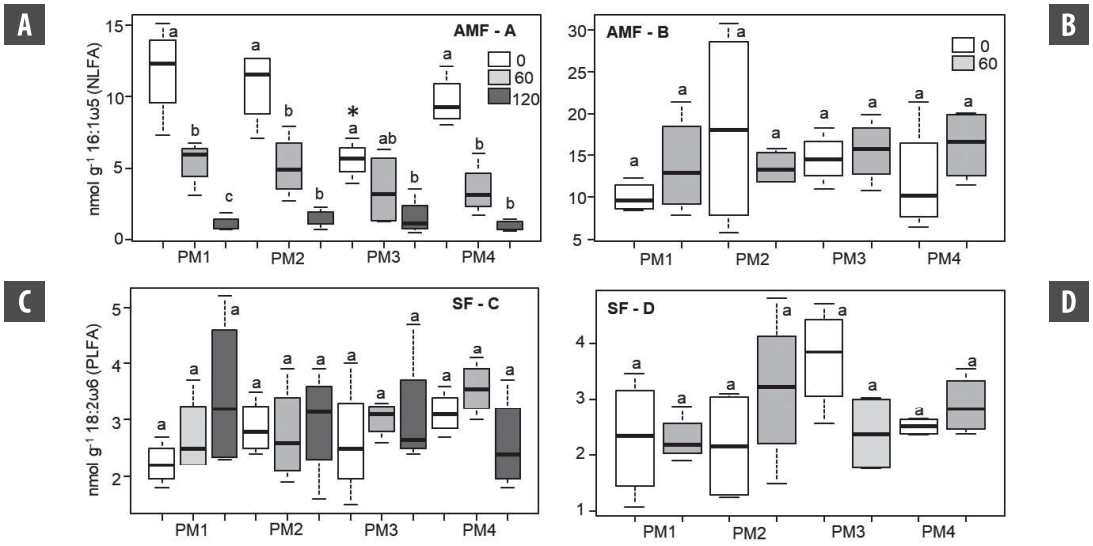

Figure 1. The amount (nmol g^{-1}) of biomarkers of arbuscular mycorrhizal fungi (AMF) and saprotrophic fungi (SF) in relation to fertilization levels (0, 60, 120 kg ha^{-1}) and plant mixtures (PM) at the Alnarp (A, C) and Lanna (B, D) experiments. Different letters and * indicate significant differences in fungal biomass under different fertilization levels and plant mixtures, respectively.

SF biomass at Lanna (R=0.38, P<0.05). This correlation could indicate the presence of P-solubilizing fungi, which constitute about 0.1-0.5% of the total fungal populations in soils (Mehta *et al.*, 2019). The different plant species mixtures did not affect fungal biomass, except for the grass-legume mixture (PM3), which had an effect only in the unfertilized plots, decreasing the AMF biomass at Alnarp (P<0.05, Figure 1A). The interactions between plant diversity and soil microorganisms are major determinant of a plant's influence on ecosystem function. Though other studies have reported that fungal biomass increases significantly with plant diversity (Eisenhauer *et al.*, 2017), such a relationship was not seen in our experiment. The site-specific response of the fungal biomass to the applied treatments might be related to the differences in soil properties or to the previous land use at the site.

Conclusions

Our findings suggest that the response of soil fungal biomass to mineral N fertilizer application has a strong site-specific component, and the reduction in the abundance of AMF, which was more sensitive than SF, only occurs under specific soil conditions.

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