Microbial biomass phosphorus and C/N/P stoichiometry in forest floor and A horizons as affected by tree species

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ABSTRACT

Forest floor horizons contain significant total P stocks, but information on the contribution of microbial biomass P (P_{MB}) and on the controlling factors of this pool is limited. Slightly modified fumigation extraction procedures were used to investigate the stoichiometric relationships of P_{MB} to microbial biomass C (C_{MB}) and microbial biomass N (N_{MB}) in the forest floor (L, F, H, and A horizons) at five sites, differing in P availability to trees, under adjacent spruce (Picea abies) and beech (Fagus sylvatica) stands. C_{MB}, N_{MB}, and P_{MB} contents were higher in forest floors under beech than under spruce. Mean stocks of P_{MB} and total P were roughly 27 and 100 kg ha^{-1} in the forest floor, respectively, but did not differ between the tree species, due to an increased organic matter accumulation in the forest floor under spruce. This reveals the importance of forest floor horizons and microbial biomass turnover for P nutrition of trees in acidic soils with the humus form moder. C/P_{MB} ratios declined from roughly 26 in L to 13 in F and H horizons, followed by an increase to roughly 17 in A horizons. The range of C/P_{MB} ratios was small at all sites in relation to the wide SOC/total P ratios of the litter used as microbial substrate, indicating a relatively strict homeostatic regulation of the forest floor microbial, mainly fungal biomass stoichiometry.

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1. Introduction

Forest floors are important P reservoirs in forest ecosystems. In the presence of moder-type humus, trees have been shown to take up considerably more phosphate from the forest floor than from the underlying mineral soil (Brandtberg et al., 2004; Jonard et al., 2009, 2010). When P-adsorbing mineral phases are lacking, the net release of plant available phosphate in forest floor horizons is determined by microbial mineralization and immobilization processes (Oberson and Joner, 2005; Rosling et al., 2016; Šantrůčková et al., 2004). Even under near steady state conditions, P is dynamically exchanged between microbial biomass and soil solution (Achat et al., 2010a, 2010c; Oehl et al., 2001). Consequently, the turnover of microbial biomass P (P_{MB}) replenishes soil solution P and contributes to P nutrition of forest trees (Achat et al., 2012).

The P_{MB} pool in temperate mineral forest soil horizons has been shown to be more closely correlated to soil organic C (SOC) or total N than to total P contents (Achat et al., 2012; Joergensen et al., 1995a,b; Khan and Joergensen, 2012). Achat et al. (2012) and Heuck et al. (2015) concluded that microbial P uptake is largely dependent on C availability, which determines the size of microbial biomass C (C_{MB}). In the forest floor, where C availability to microorganisms is considerably higher than in mineral soils, as indicated by higher C_{MB}/SOC ratios (Joergensen and Scheu, 1999a), P contents might influence P_{MB} contents more strongly. However, the few results available from studies comparing P_{MB} contents in forest floor horizons differing in total P contents are inconsistent. Saggar et al. (1998) found higher P_{MB} contents in forest floors of P fertilized Pinus radiata stands in New Zealand than in non-fertilized stands. Clarholm (1993) observed the opposite in Swedish forest floors of P fertilized and non-fertilized Picea abies stands.

C/P_{MB} and also N/P_{MB} ratios vary over a considerably wider range than C/N_{MB} ratios in temperate forest ecosystems (Chávez-Vergara et al., 2016; Joergensen et al., 1995a; Khan and Joergensen, 2012) as well as on a global scale (Cleveland and Liptzin, 2007; Hartman and Richardson, 2013; Xu et al., 2013).

According to a modeling study by Manzoni et al. (2010), the high variability of C/P$_{MB}$ ratios is one of the main reasons for the limited power of litter C/P ratios in predicting net P release rates during litter decomposition. C/P$_{MB}$ ratios decrease under high P availability, combined with low C availability and high N availability in cell culture (Anderson and Domsch, 1980; Kouno et al., 1999; Lukito et al., 1998) and incubation studies (Salamanca et al., 2006). However, there is only limited knowledge on the impact of N and especially P availability on microbial C/N/P stoichiometry in temperate forest soil ecosystems (Joergensen et al., 1995a,b). This is especially true for forest floor horizons, where C, N, and P availability strongly depends on litter stoichiometry and decomposition stage (Mooshammer et al., 2014). Relationships between the elemental composition of forest floor horizons and microbial biomass have rarely been examined, due to methodological constraints (Joergensen and Scheu, 1999b; Saggar et al., 1998). For this reason, forest floor horizons have often been removed (Yang et al., 2010; Zhao et al., 2009) or have not been analyzed (Bing et al., 2016; Yang and Zhu, 2015) in studies on P$_{MB}$ in forest ecosystems.

Microbial biomass contents and decomposition processes in forest floor horizons can be significantly affected by tree species. Different litter qualities of coniferous and deciduous species in respect to lignin and polyphenol contents, elemental composition, pH as well as physical characteristics (Berg and McClaugherty, 2014; Binkley and Giardina, 1998; Hobbie et al., 2007; Perry et al., 1987; Reich et al., 2005) have been reported to affect decomposition processes. C$_{MB}$/SOC ratios have been found to be lower under coniferous than deciduous tree species (Bauhus et al., 1998; Scheu and Parkinson, 1995; Zhong and Makeschin, 2004). Although P contents of beech leaf and spruce needle litter might be similar (Hagen-Thorn et al., 2004; Trum et al., 2011), lower C and N availability to microorganisms in spruce compared with beech forest floors may also be associated with a lower microbial P demand, resulting in lower P$_{MB}$ contents. In contrast, the higher SOC stocks in spruce forest floors (Berger and Berger, 2012; Cremer et al., 2016; Vesterdal et al., 2013) may compensate for this and result in similar forest floor P$_{MB}$ stocks.

In the present study, we investigated L, F, and H horizons as well as the upper A horizon of five adjacent beech (*Fagus sylvatica* L.) and spruce (*Picea abies* L.) stands at five sites, varying strongly in parent material and P availability. After conducting a methodological pre-study to test the effect of the type of extractant and of the soil/extractant ratio on P$_{MB}$ estimates (Bergkemper et al., 2016; Brookes et al., 1982; Khan and Joergensen, 2012), we adapted the fumigation extraction method to measure P$_{MB}$ in forest floor horizons. Our central objectives were to investigate the following hypotheses: (I) C$_{MB}$ and P$_{MB}$ contents are higher in forest floors under beech than under spruce, but there is no tree species effect on C/P$_{MB}$ ratios. (II) Stocks of P$_{MB}$ in the forest floor do not differ between tree species, due to an increased forest floor accumulation under spruce. (IIIa) P$_{MB}$ contents are affected by total P in forest floor horizons and (IIIb) by SOC contents in A horizons. (IV) C/P$_{MB}$ ratios decrease with depth from L to A horizons as the substrate is increasingly degraded.

2. Materials and methods

2.1. Study sites, soil sampling and sample preparation

Five paired forest sites, consisting of adjacent mature beech (*Fagus sylvatica* L.) and spruce (*Picea abies* L.) stands, were selected in Central and Northern Germany (Table 1). The beech stands at Vessertal and Goettingen as well as the beech and spruce stands at Solling are part of the International Co-operative Programme Forest Level II Intensive Monitoring Programme (Haussmann and Lux,

1997). The spruce stands at Vessertal and Goettingen are located 150 and 400 m apart from the respective beech plots. The beech and spruce stands at Oerrel are located on managed, non-fertilized control plots of the nutrient deficiency trial Oerrel-Lintzel, which was established in 1929 on formerly afforested heathland (Seibt et al., 1968). The beech and spruce stands at Meissner are located in the nature reserve park (Nitsche et al., 2005). Beech or spruce stands consisted of at least 90% beech or spruce trees, respectively, except for the Oerrel beech stand, which comprised 75% beech and 25% Scots pine trees.

The soils of the five sites had formed from different parent materials (Table 1) and were chosen since they represent a P availability gradient. Four of the sites were selected based on foliar P contents of the beech stands in 2013 as a measure for P availability to trees, which decreased in the order Vessertal (1.6 mg g^{-1} DM) > Solling (1.2 mg g^{-1} DM) > Oerrel (1.0 mg g^{-1} DM) $\geq$ Goettingen (1.0 mg g^{-1} DM). The Meissner site was selected due to its markedly high total P content of 1.8 mg g^{-1} soil at 0–10 cm in the A horizon, but foliar P contents were not measured (Khan and Joergensen, 2012).

Samples were taken in December 2013. At each site and stand, four sampling points were randomly selected within a 20 m × 50 m rectangular area. At each sampling point, two (for the moder profiles) to four (for the mull profile) replicate soil cores with a diameter of 8 cm were taken, using a bi-partite root auger (Eijkelkamp, Giesbeek, Netherlands). Forest floor horizons were then differentiated into intact and mainly intact litter (L horizon), moderately to strongly fragmented and fermented litter (F horizon) and, if existing, humified material without any visible litter remains (H horizon). The separated organic horizon material of each core was collected quantitatively completely to enable the calculation of stocks on a mass-per-area basis (Raubuch and Beese, 1995). The upper 5 cm of the remaining mineral soil core were separated and denominated as A horizon. Samples of each sampling point were horizon-specifically bulked and stored in polyethylene bags at 4 °C for a further 2 weeks. Prior to all analyses, L horizon material was cut with scissors to pass a 4-mm sieve. F material was passed through a 4-mm sieve without prior cutting, H and A material through a 2-mm sieve. For determining the water content and for chemical analysis (except for organic P), forest floor subsamples were dried at 80 °C and A subsamples at 105 °C for 24 h, respectively.

2.2. Microbial biomass analyses

C$_{MB}$ and microbial biomass N (N$_{MB}$) were estimated by fumigation extraction (Brookes et al., 1985; Vance et al., 1987). Field moist A horizon subsamples were split into two aliquots, equivalent to 7.5 g DM each, field moist forest floor subsamples were split into two aliquots, equivalent to 2 g DM each. One aliquot each was fumigated for 24 h at 25 °C with ethanol-free CHCl$_3$. Fumigated and non-fumigated A horizon samples were extracted with 45 ml 0.5 M K$_2$SO$_4$ by 30 min horizontal shaking at 200 rev min^{-1} and filtered (3 hw, Sartorius Stedim Biotech, Göttingen, Germany). Forest floor samples were similarly extracted with 60 ml 0.5 M K$_2$SO$_4$, corresponding to a 1 g DM to 30 ml ratio (Stockfisch et al., 1995). Organic C and total N concentrations in the extracts were measured after combustion, using a multi N/C 2100 automatic analyzer (Analytik Jena, Jena, Germany). C$_{MB}$ was calculated as E_C/k_{EC}, where E_C = (organic C extracted from fumigated samples) - (organic C extracted from non-fumigated samples) and k_{EC} = 0.45 (Wu et al., 1990). N$_{MB}$ was calculated as E_N/k_{EN}, where E_N = (total N extracted from fumigated samples) - (total N extracted from non-fumigated samples) and k_{EN} = 0.54 (Brookes et al., 1985).

P$_{MB}$ was also determined by fumigation extraction (Brookes