



Effects of long-term litter manipulation on soil carbon, nitrogen, and phosphorus in a temperate deciduous forest



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ABSTRACT

Changes in above-ground litterfall can influence below-ground biogeochemical processes in forests. In order to examine how above-ground litter inputs affect soil carbon (C), nitrogen (N) and phosphorus (P) in a temperate deciduous forest, we studied a 14-year-old small-scale litter manipulation experiment that included control, litter exclusion, and doubled litter addition at a mature *Fagus sylvatica* L. site. Total organic C (TOC), total N (TN) and total P (TP), total organic P (TOP), bioavailable inorganic P (Pi), microbial C, N and P, soil respiration and fine root biomass were analyzed in the A and in two B horizons. Our results showed that litter manipulation had no significant effect on TOC in the mineral soil. Litter addition increased the bioavailable Pi in the A horizon but had no significant effect on N in the mineral soil. Litter exclusion decreased TN and TP in the B horizon to a depth of 10 cm. In the A horizon of the litter exclusion treatment, TP, TOP and bioavailable Pi were increased, which is most likely due to the higher root biomass in this treatment. The high fine root biomass seems to have counteracted the effects of the excluded aboveground litter. In conclusion, our study indicates that aboveground litter is not an important source for C in the mineral soil and that P recycling from root litter might be more important than from above-ground litter.

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1. Introduction

Litter plays a critical role in nutrient cycling between plants and soils in forest ecosystems (Attiwill and Adams, 1993). Human disturbances and environmental changes may strongly influence forest productivity and consequently alter the above-ground litter inputs to soils. For example, forest thinning likely decreases litter inputs (Holmes et al., 2006), whereas elevated carbon dioxide (CO₂) concentration can lead to increases in productivity and litterfall (DeLucia et al., 1999; Hyvönen et al., 2007). Changes in above-ground litter inputs can have important consequences for below-ground biogeochemical processes either directly by modifying organic carbon (C) and nutrient inputs or indirectly by modifying biotic activity (Sayer, 2006). The effects of litter inputs on soil C and nitrogen (N) cycling have been studied intensively (Park and Matzner, 2003; Holub et al., 2005; Lajtha et al., 2005; Kalbitz

et al., 2007; Leff et al., 2012). However, little is known about the effect of litter inputs on soil phosphorus (P).

Litter manipulation experiments, including litter addition and litter exclusion, have often been used to examine the effects of litterfall on below-ground processes (Sayer, 2006; Sayer and Tanner, 2010). Based on a meta-analysis of 70 litter manipulation experiments, Xu et al. (2013) concluded that greater litter inputs increased soil total organic C in the top 10 cm of the mineral soil, and that soil total N in the top 10 cm decreased under litter removal, but usually was not changed by litter addition. However, there is still considerable debate concerning the contribution of above-ground litter to soil C and N. It has been reported that changing litter inputs had non-significant or only very subtle effects on surface soil C concentration (Sayer, 2006; Hoosbeek and Scarascia-Mugnozza, 2009). It has also been suggested that the responses to changing C inputs differed among ecosystems, with slower and less discernible responses in temperate forests than tropical forests (Leff et al., 2012; Xu et al., 2013). Moreover, relative to C and N, little attention has been paid to the links between litter inputs and soil P dynamics, especially in temperate ecosystems. In

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tropical forests, three years of litter removal reduced the organic P concentration in the surface 2 cm of the mineral soil by 23%, while litter addition increased it by 16% (Vincent et al., 2010). Increases in litter inputs were found to have the potential to increase the P concentration in the soil solution in tropical forests (Schreeg et al., 2013). In a lowland semi-evergreen tropical forest, Sayer and Tanner (2010) reported that soil extractable P in the mineral soil (0–10 cm) was not significantly affected by either litter addition or litter removal over the 5-year study period. As a result of the common paradigm that temperate forests appear to be limited by N rather than by P (Vitousek and Howarth, 1991), the effects of litter inputs on soil P availability in temperate forests have received little attention and are poorly understood. However, there is increasing evidence showing P limitation in temperate forests (Elser et al., 2007; Priezel et al., 2008; Braun et al., 2010; Shaw and DeForest, 2013). Therefore, it is necessary to better understand how litter inputs affect soil C, N and P dynamics in temperate forests to be able to predict temperate forest responses to environmental changes, such as elevated CO₂.

In this study, we investigated the effects of 14 years of litter manipulation on soil C, N and P as well as on the related properties such as microbial biomass, soil respiration and fine root biomass at a mature *Fagus sylvatica* L. site. We hypothesized that long-term litter exclusion decreases soil C, N and P due to the decreased inputs of C, N and P from litter, while litter addition increases them due to the higher C, N and P inputs from litter.

2. Material and methods

2.1. Study site

The litter manipulation experiment was carried out at Steinkreuz (49°52'N, 10°27'E) located in Bavaria, Germany (Park and Matzner, 2003; Kalbitz et al., 2007; Klotzbücher et al., 2013). The elevation is 460 m a.s.l. Mean annual precipitation and temperature are 750 mm and 7.5 °C, respectively. The forest is predominated by European beech trees (*F. sylvatica* L.) that are c. 140 years old. The soil is classified as Dystric Cambisol (FAO, 1998) developed from sandstone, and contains 47.2% sand, 38.8% silt and 14% clay in the A horizon (equal to the top 4–8 cm of mineral soils) and 54.7% sand, 35.2% silt and 10% clay in the B horizon. In non-manipulated plots, the mean annual production of leaf litter and its standard deviation was $519 \pm 120 \text{ g m}^{-2} \text{ yr}^{-1}$ from 2009 to 2013, and the mean annual C, N and P input from leaf litter to soils with the standard deviation was $240 \pm 58 \text{ g m}^{-2} \text{ yr}^{-1}$, $4.17 \pm 0.86 \text{ g m}^{-2} \text{ yr}^{-1}$ and $0.36 \pm 0.11 \text{ g m}^{-2} \text{ yr}^{-1}$, respectively.

2.2. Litter manipulation

The litter manipulation experiment was established in April 1999 and has been maintained ever since. Three litter manipulation treatments were each applied to four replicate plots: unchanged litter inputs (control), exclusion of above-ground litterfall (litter exclusion) and doubled above-ground litterfall (litter addition). Each plot measured 2 m × 2 m, and the distance between plots was at least 1 m. In September 1999, a roof-shaped litter trap with a wooden frame, cover net and side nets (2 m × 2 m) was placed over the plots of litter exclusion to exclude above-ground litter. The litter traps remained in the field for the entire snow-free period (April to December) in the first two years. Afterwards, the litter trap was just placed over the plots during the period of main litterfall (from September to December) in order to keep the plots as little disturbed as possible (Kalbitz et al., 2007). All excluded litter except for woody debris with a diameter of >1 cm was removed and redistributed on the litter addition plots. The litter exclusion led to

the disappearance of the litter layer within the first year of the experiment (Karsten Kalbitz: personal communication). As the cover net (pore size < 5 mm) was evaluated to have minimal water retention by pre-installment tests (measuring percent recovery of water sprayed over the net), only a small portion (<5%) of throughfall under normal rainfall events has been excluded by the litter trap itself (Park and Matzner, 2003). Temperature logger that was installed in spring 2000 and measured temperature at an interval of 30 min showed that the soil temperature was not changed by the treatments (Kalbitz et al., 2007).

2.3. Soil sampling and fine root biomass determination

Soils were sampled in October 2013. Three soil cores (8.5 cm in diameter) per plot were randomly taken and divided into three horizons in the field: A horizon (equal to the top 4–8 cm of mineral soils), B horizon to a depth of 10 cm and B horizon 10–20 cm depth. Samples from the same soil horizon were combined to gain one mixed sample per plot and horizon. Volumetric sample were taken in order to determine the bulk density. The sieved samples (<2 mm) were stored for less than five days at 5 °C until analysis of microbial biomass and activity. Soils were air dried for analysis of soil chemical properties and soil P fractions. Fine roots (<2 mm) collected from soils were oven-dried at 40 °C until constant weight was obtained. No further separation into living or dead root mass was done.

2.4. Soil chemical analyses

Soil pH was determined using a soil-to-water ratio of 1:2.5. Total organic C (TOC) and total N (TN) were measured by an element analyzer (Vario EL, Elementar Analysensysteme, Hanau, Germany). Inorganic P (Pi) extracted with anion exchange resin (resin-Pi) and by NaHCO₃ (NaHCO₃-Pi) was measured to gain insights into the bioavailable Pi fractions according to Hedley et al. (1982). For this purpose, 0.5 g of air-dried soil was extracted with water in the presence of an anion exchange membrane on a horizontal shaker. After 16 h, the anion exchange membrane was transferred to 25 mL of 0.5 M HCl to re-dissolve Pi. Subsequently, soil samples were extracted with 25 mL 0.5 M NaHCO₃ (pH 8.5) on a horizontal shaker in order to determine NaHCO₃-extractable Pi in soil. After 16 h, the solution was centrifuged to collect supernatant for Pi analysis. The concentrations of Pi in both extracts were measured colorimetrically by a flow injection photometer (FIA compact, MLE, Dresden, Germany).

The concentrations of soil TP, as the sum of total Pi (TPi) and total organic P (TOP), were determined by the ignition method (Saunders and Williams, 1955). That is, 0.5 g of air-dried soil was ignited at 450 °C for 16 h. The ignited and a similar amount of non-ignited soils were extracted with 25 mL 0.5 M H₂SO₄ on a horizontal shaker for 16 h. The concentrations of TPi in the extracts were measured colorimetrically by a flow injection photometer (FIA compact, MLE, Dresden, Germany). The concentration of TOP was determined by the difference in extractable Pi between the ignited and the non-ignited samples.

2.5. Microbial biomass carbon, nitrogen and phosphorus

Soil microbial biomass carbon (MC), soil microbial biomass nitrogen (MN) and soil microbial biomass phosphorus (MP) were determined by the fumigation-extraction method (Brookes et al., 1982, 1985; Vance et al., 1987;). Organic C and TN extracted with 0.5 M K₂SO₄ in fumigated and non-fumigated soil samples were measured using a CNS analyzer (Vario EL, Elementar Analysensysteme, Hanau, Germany). MC and MN were calculated with a

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