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Is there a convergence of deciduous leaf litter stoichiometry, biochemistry and microbial population during decay?

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ABSTRACT

Litter decomposition is driven by saprotrophic microbial communities. The structure of this community varies over the course of decomposition, which has been linked to the chemistry of litter resources. Here, we test the hypothesis that leaf litter from different tree species becomes more similar in terms of litter stoichiometry, C-biochemistry during the course of decomposition (convergence).

We also test if these variations can be linked to shifts in microbial community structure and abundance. For this reason, leaf litter of four deciduous tree species — beech (*Fagus sylvatica*), oak (*Quercus robur*), alder (*Alnus glutinosa*) and ash (*Fraxinus excelsior*) were sampled from a temperate forest in February, May and September 2010. We measured variations in leaf litter stoichiometry by elemental concentrations, C-biochemistry with Fourier transform infrared (FTIR) spectroscopy and litter microbial community structure and abundance with phospholipid fatty acid (PLFA) analysis. For litter from beech, oak and ash, C:N and C:P ratio converged with time when exposed to the same environmental conditions during degradation. In contrast, C:nutrient ratios increased in alder litter over time. Litter C-biochemistry was to a large extent related to shifts in C:N ratios. Principal component analysis PCA of FTIR bands, revealed that divergence in C-biochemistry over the course of decomposition was dependent on litter type, and hence, with initial C:N ratio. Litter-decomposing bacterial community was highly relevant to C-biochemistry and environmental conditions (temperature and moisture) while fungi community was more nutrients-related.

In conclusion, initially wide C:nutrient ratios tend to converge during decay; while nutrient limitation may lead to divergence of stoichiometry. In general, there was a trend towards divergence of C-biochemistry and microbial community structure.

1. Introduction

Mineralization of freshly senesced leaf litter by decomposer communities generates one of the greatest C fluxes in the global C cycle (Schlesinger and Andrews, 2000) and returns nutrients back to soil, which makes them available to plant growth. Furthermore, microbes themselves have nutrient demands for their energy- and growth-related metabolism and thereby affect the decomposition process. Decomposer microorganisms have smaller C:nutrient ratios (C:N, C:P) than the organic substrates they consume (Cleveland and Liptzin 2007; Sterner and Elser, 2002), which is known as "stoichiometric imbalance" (Mooshammer et al., 2014; Zechmeister-Boltenstern et al., 2015). The stoichiometric difference between microbial communities and organic substrates generally decreases from leaf litter to topsoil to subsoil organic matter during decay. Exemplified by the global temperate forest C:N ratio, the stoichiometric imbalance (calculated on mass basis ratio of $C:N_{resource}$ over $C:N_{microbes}$) declined from ~7 in leaf litter to ~3 in soil organic matter (Xu et al., 2013).

It has been proposed that litter chemistry also converges towards a common chemistry regardless of any taxonomic variation among soil microbial and faunal communities (Fierer et al., 2009). Leaf litter C-biochemical compounds (C-biochemistry) vary in their susceptibility to decomposition, on a spectrum from labile molecular such as carbohydrates and amino acids to more complex carbon compounds (e.g., polysaccharides, lignin). So, if litter stochiometries converge, the litter C-biochemistry convergence may also exist. Research on litter C-biochemistry similarity has been largely facilitated by information provided by ^{13}C nuclear magnetic resonance (NMR) and by Fourier transform infrared (FTIR) spectroscopy. Preston et al. (2009) found a muting of NMR spectroscopy peaks of 11 foliar litter samples as decomposition proceeded, which could be interpreted as the convergence of C-biochemistries. Tatzber et al. (2011) showed that organic and mineral soil layers in forest soils can be distinguished along their C-biochemistry. The organic layers showed a better discrimination in FTIR bands, which decreased with humification indicating a convergence with decomposition (Tatzber et al., 2011).

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The C-biochemistry of leaf litter varies among litter types (such as the proportions of labile and recalcitrant C-compounds) and these litter-derived C-compounds/substrates can be utilized by a wide range of microorganisms (Suseela et al., 2013) and conversely microorganisms also adapt to their substrate (litter) over the course of decomposition. Therefore, there may also be converging trend for microbial community structure and abundance during litter decomposition. Many studies have shown strong correlations between microbial community structure and a range of factors such as C quantity and quality, pH as well as C:N ratio (Fierer and Jackson, 2006; Lozupone and Knight, 2005; Nemergut et al., 2010). Additionally, we included C-biochemistry, C:N and C:P ratios, litter nutrients (K, Mg, Ca, Zn, Mn), as well as environmental factors — temperature and moisture, in order to explore the convergence on multiple-levels – stoichiometry (elemental ratios), C-biochemistry (C-containing functional groups characterized by FTIR spectroscopy) as well as microbial community structure and abundance (measured via PLFA). We report for the first time the combination of FTIR data, PLFA analysis together with stoichiometry in a set of various leaf litter types. We hypothesized that (H1) leaf litter stoichiometry (C:N and C:P) converges during decay, so C:nutrient ratios will decline from February to September; that (H2) leaf litter C-biochemistry will also converge and will be correlated with shifts in litter stoichiometry; and (H3) litter stoichiometry and C-biochemistry will have greater impacts on microbial communities than will environmental factors, leading to the convergence of litter inhabiting microbial communities. To this end we investigated four deciduous leaf litter types – beech (*Fagus sylvatica*), oak (*Quercus robur*), alder (*Alnus glutinosa*) and ash (*Fraxinus excelsior*) – in February, May and September 2010, to identify whether litter becomes similar during decay in terms of stoichiometry, leaf litter C-biochemistry as well as decomposing microbial community structure and abundance.

2. Materials and methods

2.1. Site

Four deciduous leaf litter types were collected from the northern Vienna Forest (Schottenwald, Austria; (Kitzler et al., 2006)), which is located in direct vicinity of Vienna 48°10′60″ N and 16°13′60″ E 386 m asl, and has a mean annual temperature of 9 °C and a mean annual precipitation of 465 mm. Beech, oak, alder and ash litter was sampled on 10th February, 31st May and 14th September 2010. Soil in the study area is a Dystric Cambisol over Flysch rocks from the Rhenodanubian Flysch zone, which is a part of the Penninic super unit at the borderline of the “Laberer” nappe and the “Kahlenberger” nappe. Soil C:N is 16 and a pH of 4.4 measured in $CaCl_2$ solution (Kitzler et al., 2006). Soil is classified as a silty loam with a texture of sand 28.3%, silt 44.6% and clay 27.1%.

2.2. Litter sampling

Approximately 500 g of fresh weight leaves of each individual litter was sampled at pure tree species stands that were close to each other (<500 m distance) so that the same climate conditions prevailed. Three biological replicates were collected from three plots (10 × 10 m) within an area of 50 × 50 m. Only the L-horizon was sampled, carefully excluding mineral soil material and O-horizon. The litter was transported to the laboratory at ambient temperature, mixed thoroughly and cut into <0.5 cm^2 pieces using a Retsch mill (MM2000, Haan, Germany). Aliquots of the three biological replicates per sampling site were stored at −20 °C until further processing.

2.3. Determination of litter nutrient quality

Water content (WC) was determined gravimetrically by drying the litter at 105 °C for 24 h. Litter pH was measured in 25 ml 0.01 M $CaCl_2$ with 2 g of field moist litter, based on Austrian standards for soil samples (ÖNORM L1083, 2005). For elemental analysis, aliquots of dry litter samples were ground in a mill (Retsch MM2000, Hanau, Germany) to a fine homogeneous powder. Total carbon (C) and nitrogen (N) contents of the litter were analyzed with an elemental analyzer (Leco CN2000, LECO corp. St Joseph, MI, USA) (Schneider et al., 2012). Phosphorous (P), potassium (K), magnesium (Mg), manganese (Mn), calcium (Ca), iron (Fe) were determined by inductively coupled plasma atomic emission spectrometry (ICP-AES) after acid hydrolysis (H_2SO_4 + HNO_3) in a microwave oven (CEM MARS Express) (Henschler, 1988). These nutrient concentrations were used to calculate leaf litter C:N, C:P and N:P ratios (all ratios given are on a mass basis).

2.4. PLFA analysis

Lipids were extracted from 1 g field moist sub-samples using a modified Bligh and Dyer technique (Hackl et al., 2005). Extracted PLFAs were analyzed with a HP 6890 Series GC-System connected to a 7683 series injector and auto sampler on a HP-5 capillary column and detected with a flame ionization detector. For identification of the fatty acid methyl esters, an external standard (bacterial acid methyl ester mix from SUPELCO) was used. For quantification of the peaks, methyl nonadecanoate fatty acid (19:0) was added as an internal standard (for more details see Brandstätter et al., 2013). PLFA nomenclature used was that described by Frostegård et al. (1993). The ratio fungal/bacterial PLFA was calculated by the amount of total bacterial PLFAs divided by the sum of 18:2ω6 and 18:1ω9 (Frostegård and Bååth, 1996). The iso- and anteiso-branched saturated fatty acids (i14:0, i15:0, a15:0, i16:0, i17:0, a17:0) represent Gram-positive bacteria (Zelles et al., 1994), whereas cyclopropyl (cy17:0, cy19:0), the monounsaturated 16:1ω7c and the straight chain fatty acids 14:0, 15:0, 17:0 represent Gram-negative bacteria (Kourtev et al., 2002). We calculated the sum of the PLFAs i14:0, i15:0, a15:0, i16:0, i17:0, a17:0, 14:0, 15:0, 16:1ω7c, cy17:0, 17:0, cy19:0, 10Me16:0 and 10Me17:0 as an index of bacterial biomass (Frostegård and Bååth, 1996). The PLFAs 18:2ω6,9 and 18:1ω9c were used as indicators for fungal biomass (Olsson, 1999). 10Me18:0 was as indicator of actinobacteria (Kroppenstedt, 1985).

2.5. FTIR analyses

FTIR analyses of litter samples were done using potassium bromide (KBr) pellets, which were produced by mixing an exact amount of 2 mg ground dry litter sample with pure KBr (FTIR grade) to reach a total weight of 200 mg before being grinded and homogenized in a vibrating ball mill (Retsch MM 200, Haan, Germany) for 30 s. This mixture was hydraulically pressed under vacuum at 10 tons for 3 s to obtain a transparent pellet, and then stored in a desiccator until the FTIR analysis. The mid-infrared spectra were recorded with a Bruker Tensor 27 Spectrometer in the mid infrared area (400 to 4000 cm^{-1}). The recordings were carried out with a resolution of 4 cm^{-1}, weak apodization and 16 scans per sample. Background correction against ambient air and pure KBr was carried out by every 12 samples (Tatzber et al., 2007). The software used for band integrations was “OPUS 6.5”. Band areas were integrated (after transformation of the spectra from transmission into absorbance units) with corrected baselines; the obtained units were A cm^{-1} (“absorbance; wavenumber units”). These band areas were normalized by dividing the band area by the weighed amount of analytes in the pellet, leading to the unit A cm^{-1} mg^{-1}. Detailed band interpretations are based on assignments of FTIR bands to functional groups as listed in Table 2. (Boeriu et al., 2004; Tatzber et al., 2007).

2.6. Statistical analyses

Variables were tested for normal distribution by the Shapiro–Wilk test and homogeneity of variance between groups as assessed by