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Tree species identity influences the vertical distribution of labile and recalcitrant carbon in a temperate deciduous forest soil

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ABSTRACT

In terrestrial environments, soil organic matter (SOM) is the largest organic carbon (C) pool. The quantity and quality of organic carbon in soils can be affected by vegetation through influencing the inputs and outputs of SOM. We examined how storage and quality of C in SOM were affected by vegetation under grass cover or single and a polyculture plot of *Betula pendula*, *Alnus glutinosa* and *Fagus sylvatica*. An acid hydrolysis approach was used to quantify three SOM fractions differing in biodegradability. Tree species identity and stand composition had no significant effect on the total amount of C stored in different SOM fractions to a depth of one meter. However, when examining individual SOM fractions in the upper layers of the soil profile, significantly more C was stored in the putatively more labile fractions 1 and 2 under *F. sylvatica* and *A. glutinosa*, respectively. In deeper soil layers, the highest storage of recalcitrant organic C was found under the tree polyculture. The vertical distribution of these three soil organic C pools was compared to C inputs via decomposed leaf litter. Our data indicated that in the tree species polyculture, combining litter inputs of multiple species can have a positive impact on the accumulation of acid resistant recalcitrant C in deep soil layers in 4 years. This C fraction has the greatest potential for long-term sequestration.

1. Introduction

Soil organic matter (SOM) represents the largest reservoir of terrestrial organic carbon (C) on Earth, and the organic residues that comprise SOM range from relatively intact plant or microbial material to highly decomposed humic substances (Rumpel et al., 2002). Through differences in litter quality, plant species have potential to influence the storage and dynamics of C in soils, as reported in several previous studies (Binkley and Valentine, 1991; Hagen-Thorn et al., 2004; Jandl et al., 2007; Leuschner et al., 2013). However, there is growing evidence that molecular structure is less important than previously believed as a factor controlling the formation of SOM, and that the inputs from roots and microbial degradation products are more important than previously assumed (Schmidt et al., 2011). However, it remains undisputed that these factors result in a heterogeneous mixture of organic compounds (von Lützow et al., 2006; Schmidt et al., 2011; Tfaily et al., 2015).

To investigate the composition of soil organic matter, SOM can be fractionated into several pools using a range of techniques (eg. Paul et al., 2001; Weil et al., 2003; Gregorich et al., 2003; Bajgai et al., 2013). Each of these methods attempts to isolate soil organic matter pools of different longevity. A common method used, is to separate pools on the basis of biodegradability (Rovira and Vallejo, 2007). This method separates soil organic matter based on acid solubility into labile and recalcitrant pools, which are believed to have different turnover times (McLauchlan and Hobbie, 2004). For example, labile C fraction composed of compounds such as soluble sugars, starch, and other carbohydrates, has been show to play a dominant role in the evolution of CO_2 from soil due to preferential decomposition and rapid turnover (Belay-Tedla et al., 2009). In contrast the recalcitrant fraction is thought to degrade slowly, thus contributing to long term C storage in soils. For example, lignified humus and some physically protected labile SOM can be retained in soils for several thousand years (Zou et al., 2005; Dungait et al., 2012; Kellner et al., 2014). However, how the composition of aboveground vegetation affects the distribution of these pools in soil systems is largely unknown (De Deyn et al., 2008).

Tree species may influence soil organic carbon (SOC) stocks through a variety of mechanisms such as: (i) differences in net primary productivity and the production of detritus (Montagnini et al., 1993; Hansen et al., 2009), (ii) variation in the quality and

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complexity of organic matter input to soils detritus originating from leaf, root and mycorrhizal biomass (Hagen-Thorn et al., 2004), (iii) variation in the depth and distribution of roots (Carvalheiro and Nepstad, 1996; Lai et al., 2015), and (iv) by altering soil invertebrate and microbial populations (Hobbie et al., 2006; Lynch et al., 2012). Soil organic carbon accumulation may differ in species–diverse communities, compared to monocultures through variation in biomass inputs to soils, and SOM transformation processes in the soil (Steinbeiss et al., 2008). In mixed species communities, species interactions may either increase productivity through resource use complementarity (Loreau and Hector, 2001; Richards and Schmidt, 2010) or decrease productivity through inter- or intra-specific competition (Carnus et al., 2006). Plant community composition in mixtures may also influence long term storage of soil C through species–specific differences in plant detritus chemical composition and input rates (Six et al., 2002). In addition, interaction between litter types in mixed species communities may affect rates of decomposition and turnover (King et al., 2002). This is particularly true in natural forest ecosystems, where the litter layer can be comprised of inputs from many species, thus forming more complex organic substances compared to the litter layer of mono-specific forests. The highly complex and heterogeneous organic residues found in the SOM of mixed communities have been shown to alter soil C residence times through differences in biodegradability (Sollins et al., 1996).

Our study determined the vertical distribution of labile and recalcitrant C fractions in SOM that occurred within the initial four years of forest establishment. We examined the effects of vegetation on the storage of C fractions in soils under grass or trees of *Alnus glutinosa*, *Betula pendula*, *Fagus sylvatica* grown in monoculture, or a polyculture of the three tree species. *B. pendula* is a light-demanding, early successional species with fast juvenile growth (Fischer et al., 2002). *A. glutinosa* is an N-fixing, water-demanding pioneer species, also with high juvenile growth rates (Braun, 1974). Lastly, *F. sylvatica* is shade tolerant and slow growing when juvenile (Ellenberg et al., 1991), can persist in the understory, and often dominates late successional forest. We hypothesized that in the long term SOC storage would be positively affected by growing trees species selected for contrasting functional traits in polyculture.

2. Materials and methods

2.1. Study area

The BangorDIVERSE experimental site was established at Henfaes Research Centre, Bangor University, North Wales, UK (53°14′ N, 4°01′W) in March 2004 on two fields with a total area of 2.36 ha. Soils are fine loamy brown earth over gravel (Rheidol series) and classified as Fluventic Dystrochrept in the USDA system (Smith et al., 2013a). Soil texture in the 0–10 cm layer was 48.2 ± 1.3% sand, 33.6 ± 0.9% silt and 18.2 ± 2.1% clay, determined by laser diffraction (Coulter LS particle size analyser). The soil pH is 5.4 in the 0–10 cm layer increases to 6.3 at 100 cm soil depth. Soil physicochemical properties are shown in Suppl. Table 1. Climate at the site is classified as hyperoceanic. Mean annual temperature throughout 2005–2008 was 11.5 °C with an annual rainfall of 1034 mm (Campbell Scientific Ltd, Shepshed, UK).

2.2. Plantation design

Before tree planting, all vegetation was removed from the fields including the grass plots and the soil was ploughed and raked. Plots were established in four replicated blocks of single species or two and three species mixtures of *A. glutinosa* L., *B. pendula* Roth., *F. sylvatica* L., *Fraxinus excelsior*, *Acer pseudoplatanus* L., *Castanea sativa* Mill. and *Quercus robur* L. The trees were selected due to their contrasting shade tolerance, successional chronology and to represent a range of taxonomic, physiological and ecological types. Two blocks were sited in each field and the minimum distance between any two plots of the same composition was 35 m. The size of the plots were 62 m^2 for the grassland, 81 m^2 for single species, and 121 and 196 m^2 for the two species and three species plots respectively. A replacement series design (with inter-tree spacing constant between treatments) was selected because of the experiments objective of being realistic in reflecting the practical realities of how forests comprising monocultures or mixtures of potential canopy tree species could be established (Jolliffe, 2000). The site was planted with 60 cm saplings of each species with an inter-tree spacing of 1 m (10,000 stems ha^{-1}). A systematic hexagonal planting design (Aguiar et al., 2001) was used to maximise the mixing effect so that, in the three species mixture blocks, each tree was surrounded by nearest neighbours of two-con-specific individuals and one and three individuals of the other two species, respectively, resulting in each tree having six equidistant neighbours. On the grassland plots, a grass cover was allowed to regenerate from remnants to form a sward composed of a mixture of *Lolium perenne* L., *Dactylis glomerata* L. and *Agrostis stolonifera* L. In the work reported here, we used the plots of *A. glutinosa*, *B. pendula* and *F. sylvatica* and a three species polyculture of these species.

2.3. Positioning of the plots used

The initial soil organic matter was determined in the top 0–10 cm layer on a 10 × 10 m grid. The mean SOM content across the fields was ca. 6%, but across the fields varied between 4% and 8% (Fig. 1). Historically, both fields were pasture, but since the 1980s one field (field 1) was used for small scale forestry experiments, while in 2003 the other field (field 2) was ploughed and planted with oil seed rape. Two blocks each containing a replicate single species and mixed species plot as well as the associated grassland plot, were located on the field used in 2003 for soil seed rape. In the other field one block was positioned on an area previously planted with *Salix* as short rotation coppice trial, while the other block was positioned on an area previously used to grow mainly *Q. robur* and *F. sylvatica* saplings. In 2008, one of the *A. glutinosa* plots was damaged. To balance the number of replicates, one plot each of the *F. sylvatica*, *B. pendula* and polyculture were removed from the analysis by random selection, leaving the distribution of plots used as shown in Fig. 1.

2.4. Soil collection and sample processing

Soil was collected by excavating 100 × 100 × 100 cm pits in the centre of each grassland plot and each of the three tree species monoculture and tree three species polyculture plots in September of 2008 (15 pits in total). In the polyculture plots, samples were collected from a pit at an equal distance from *A. glutinosa*, *B. pendula* and *F. sylvatica.* Soil samples were collected from seven layers (0–10, 10–20, 20–30, 30–40, 40–50, 70–80 and 90–100 cm). A subset of four layers (0–10, 10–20, 40–50 and 90–100 cm) were then used for C fractionation using acid hydrolysis. To ensure representativeness, samples were obtained from each layer, approximately 100 g of soil was collected from each side of the soil pit and mixed to produce a composite 400 g sample. Soils were then air dried, carefully homogenized and sieved to pass through a 2 mm sieve, a 50 g sub-sample was then taken and finely ground using a ball mill (Retsch Mixer Mill MM 200) and passed through a 100 μm sieve prior to acid hydrolysis. Soil pH and electrical conductivity were determined in a 1:2 v/v slurry of soil and distilled water with standard electrodes. Moisture content was

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