



Short communication

What controls the concentration of various aliphatic lipids in soil?

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ABSTRACT

The composition of lipids in soil offers clues to the origin and stabilization of soil organic matter, but the descriptive nature of prior research makes quantitative interpretations problematic. We statistically evaluated potential predictors of the concentrations of aliphatic lipids in mineral soils beneath plantations of 11 tree species. Lipids were recovered from leaves, roots, and soils from each plantation using base hydrolysis and solvent extraction. Nearly 70% of the variation in individual soil lipid concentrations was explained by lipid concentrations in tree leaves and roots. Less variation in soil lipid concentrations was attributed to lipid properties such as functional group composition, chain length, and whether a lipid was most abundant in leaves or roots. Surprisingly, although the chemical and biological compositions of soils were highly variable for plantations of different tree species, the tree species identity had little impact on soil lipid concentrations and the effects of lipid properties were similar for all plantations.

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Main text

There is considerable variability in the abundance and composition of extractable and hydrolyzable lipids in soil (Bull et al., 2000; Jansen et al., 2006; Naafs et al., 2004; Nierop et al., 2006; Otto et al., 2005; Quenea et al., 2004; Rumpel et al., 2002), but it is difficult to quantitatively and causally relate this variability to properties of lipids and soils that influence organic matter stabilization. For example, mineral soils often have relatively high concentrations of plant-derived lipids that are abundant in roots but not in leaves (Mueller et al., 2012b), but the causes of this pattern are not clear. Does this pattern arise because lipids present in roots (i.e. "root lipids") have different chemical properties, such as lipid chain

length or chemical functional groups (Fig. 1) that influence lipid stabilization in soil? Or, does this pattern arise because root-derived biomolecules are preferentially stabilized in soils, regardless of their biochemical properties (Rasse et al., 2005)? Conversely, could the abundance of root-derived lipids in soils reflect an effect of soil biota, such as preferential consumption of leaf litter by anecic earthworms (Curry and Schmidt, 2006)? To address these questions and, more generally, to achieve a mechanistic understanding of soil lipid composition, studies and statistical analyses must be designed to quantify the effects of multiple factors, including lipid origin, lipid biochemistry, and various soil properties. The concentrations of individual lipids in their sources must also be accounted, otherwise selective preservation or degradation cannot be identified or quantified. Yet, such quantitative analyses have not been conducted previously.

Here, we statistically evaluate potential controls of soil lipid concentrations beneath tree plantations in central Poland (51°14.87'N, 18°06.35'E). In 2008, we sampled mineral soils (0–20 cm) in large plots of 11 temperate tree species (2–6 plots each

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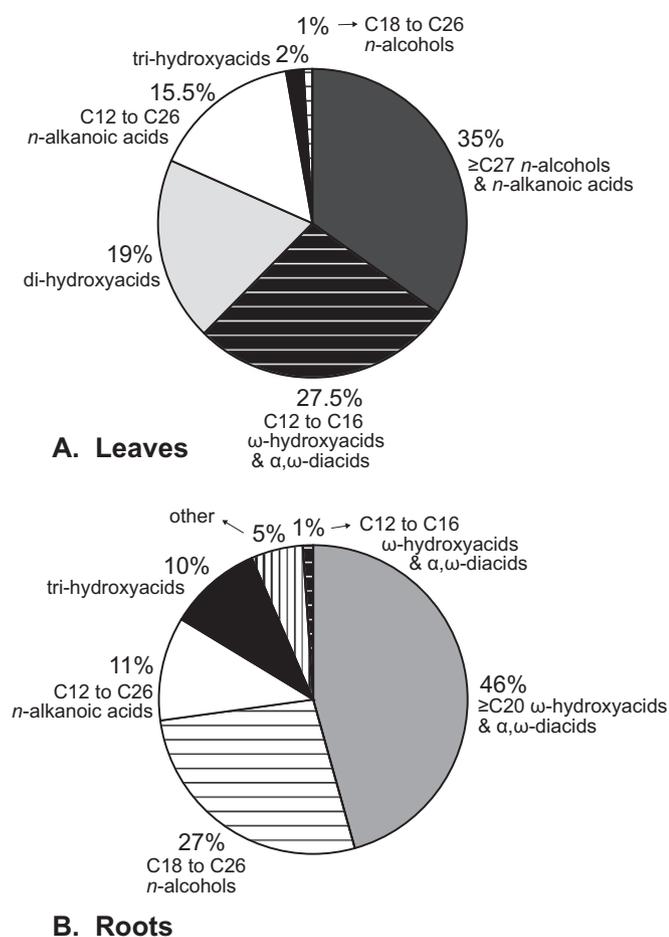


Fig. 1. The lipid chain lengths and lipid types, as defined by chemical functional groups, for aliphatic lipids that were present in soil and predominately derived from either leaves (A) or roots (B). Lipids were defined as predominately derived from leaves or from roots if they were at least 10 times more abundant in one plant organ relative to the other. Accordingly, 109 lipids were predominately in leaves and 92 lipids were predominately in roots. Each percentage is an average of data from 11 different tree species monocultures.

for *Abies alba*, *Acer platanoides*, *A. pseudoplatanus*, *Fagus sylvatica*, *Larix decidua*, *Pinus nigra*, *P. sylvestris*, *Pseudotsuga menziesii*, *Quercus robur*, *Q. rubra*, and *Tilia cordata*). Plots were planted as monocultures in 1970 and 1971 following clear-cutting of an 80-year-old *P. sylvestris* plantation and plowing to a depth of 30–60 cm. Thus, during the 118 years prior to sampling, the majority of plant inputs in a given plantation could be accounted for by *P. sylvestris* and the subsequently planted tree species. Surface soils are derived from sandy glacial outwash; with one exception, the top 20 cm of mineral soil in all plots is >70% sand and <10% clay (mostly vermiculite and kaolinite). Notably, plantations of different tree species are widely divergent with respect to soil chemistry and the composition of soil biota (Hobbie et al., 2006; Mueller et al., 2012a; Reich et al., 2005), two factors that could influence the composition of soil lipids (Bull et al., 2000; Crow et al., 2009; Nierop and Verstraten, 2003).

Lipids from soil samples (sieved to 2 mm), green leaves, and fine roots (<2 mm diameter) of each tree species were recovered in hexane and ethyl acetate extracts following hydrolysis with methanolic potassium hydroxide. Derivatized lipids were identified and quantified by GC–MS using extracted ions associated with each analyte and those of 32 external standards. Focal lipids included a range of *n*-alkanoic acids (C₁₂ up to C₃₀), α,ω-diacids (C₉ up to C₂₆),

n-alcohols (C₁₂ up to C₃₀), ω-hydroxyacids (C₈ up to C₂₆), and di- and trihydroxyacids, encompassing monomers of cutin, suberin, and plant waxes (Kolattukudy, 2001). Averaged across all plantations, the total masses of quantified lipids in leaves, roots, and soils were 39.9, 35.4, and 29.0 mg g⁻¹ C. Experimental and procedural details and a list of analytes were shown in Mueller et al. (2012b). Statistical analyses were performed using log-transformed lipid concentrations and type III sums of squares, such that the explanatory power of each predictor was evaluated *after* accounting for effects of the other predictors (Hector et al., 2010).

According to multiple regression (Fig. 2), nearly 70% of the variability in soil lipid concentrations could be attributed to two factors: (i) the concentrations of lipids in leaves and roots of the tree species *currently present* in each plantation and (ii) the concentrations of lipids in leaves and roots of the tree species that *previously occupied* all plots (*P. sylvestris*). In one-factor models, each of these predictors alone accounted for ca. 55% of the variability in soil lipid concentrations (Table 1; some explanatory power was shared because the two predictors were positively correlated: $P < 0.0001$, $R^2 = 0.42$, $n = 379$). Thus, some of the extractable and hydrolyzable lipids in soils are likely derived from *P. sylvestris* trees that have been absent for nearly 40 years. The very strong relationship between the concentrations of lipids in soil and in plant tissues suggests that extractable and hydrolyzable plant lipids are retained in soil by a process that operates somewhat independently of lipid chemical properties and origin (e.g. occlusion of plant litter within soil aggregates).

To assess whether other factors could account for variation in soil lipid concentrations that was not explained by leaf and root lipid concentrations, we added the following predictors to the model: the location and type of chemical functional groups (*lipid type*), the number of carbon atoms in each lipid (*chain length*), the

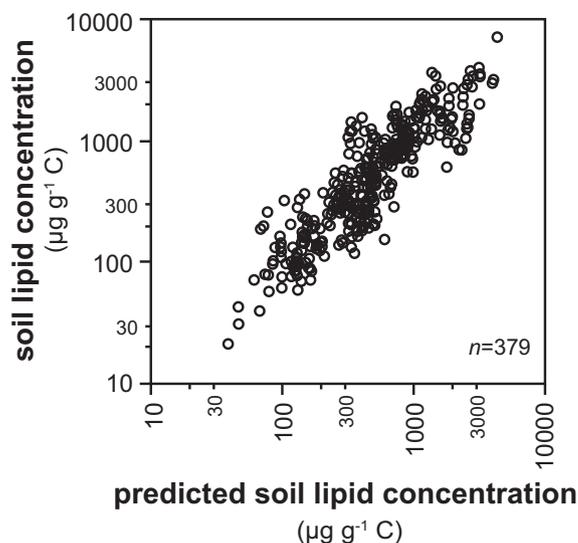


Fig. 2. Biplot of actual soil lipid concentrations (Y-axis) vs. soil lipid concentrations predicted by a multiple regression model that contained two factors: (i) the sum of lipid concentrations in leaves and roots of the tree species *currently present* in each plantation and (ii) the sum of lipid concentrations in leaves and roots of the tree species that *previously occupied* all plantation plots (*Pinus sylvestris*). Note the log₁₀ scale on the X and Y-axes. The regression model explained 69% of the variability in untransformed soil lipid concentrations (according to a linear fit of the actual values vs. the predicted values). Each circle represents a single lipid that was observed in soil and in tree leaves or roots from the present and previous plantations. Each lipid could be represented up to 10 times, once for each tree species. Soil lipid data from the current *P. sylvestris* plantation were excluded. We also excluded data in 36 instances for which a lipid observed in soil was not quantified in plant tissues of both the current plantation species and *P. sylvestris*.

Table 1
Proportion of variance explained by different predictors of soil lipid concentrations ($n = 379$ for each model).

Predictor	R^2 from models with only one predictor (for log-transformed Y)	R^2 from models with only one predictor ^a (for untransformed Y)	Partial R^2 from model with all six predictors ^b (for log-transformed Y)	P value from model with all six predictors
Lipid concentration in leaves and roots of <i>present</i> plantations ($\mu\text{g g}^{-1}\text{C}$)	0.66	0.55	0.09	<0.0001
Lipid concentration in leaves and roots of <i>previous</i> plantation ($\mu\text{g g}^{-1}\text{C}$)	0.64	0.56 ^c	0.09	<0.0001
Lipid type (as defined by chemical functional groups)	0.11	0.11	0.06	<0.0001
Lipid carbon chain length (number of carbon atoms)	0.09	0.02 ^c	0.03	<0.0001
Proportion root-derived (=root conc./leaf + root conc.)	0.01	0.001 ^c	0.005	0.0002
Tree species identity in <i>present</i> plantations	0.01	0.004	0.009	0.001

^a R^2 values were estimated by fitting the untransformed soil lipid concentrations with the predicted values from corresponding one-factor ANOVA models of log-transformed soil lipid concentrations.

^b Partial R^2 values were estimated by dividing the Type III sums of squares for each factor (from the model with all six predictors) by the total sums of squares for the log-transformed soil lipid concentrations. Thus, partial R^2 values indicate the proportion of variance in log-transformed soil lipid concentrations that could be uniquely explained by each factor. Partial R^2 values do not sum to give the R^2 value of the whole model because of covariance among model predictors (Hector et al. 2010).

^c Lack of fit test was significant ($P < 0.05$) for the linear regression between predicted and untransformed soil lipid concentrations, so the noted R^2 value should be interpreted with caution.

relative abundance of lipids in leaves and roots of each tree species (*lipid origin*, calculated by dividing the lipid concentration in roots by the sum of its concentration in leaves and roots) and the identity of the tree species in the present plantations (*tree species*). Each of these factors explained variation not accounted for by leaf and root lipid concentrations. However, individually, none of these additional factors explained more than 11% of the variation in soil lipid concentrations (Table 1). Collectively, lipid type, chain length, lipid origin, tree species, and lipid concentrations in leaves and roots accounted for 83% of the variation in soil lipid concentrations.

The estimated effects of lipid type, chain length, and lipid origin must be carefully interpreted because we did not account for lipid concentrations in other sources (e.g. bark, seeds, microbes, understory plants). Thus, for example, an apparent positive effect of chain length on soil lipid concentrations could arise if long-chain lipids in soil are substantially derived from those uncharacterized sources. However, confounding effects of unaccounted sources are likely minimal because: (1) dead leaves and roots typically account for the majority of organic inputs to soil (Fahey et al., 2005), (2) all focal lipids were identified in leaves and/or roots of the overlying trees, (3) understory vegetation is minimal in these plantations (M. Kasproicz unpublished), and (4) most of the focal lipids (Fig. 1) are uncommon in microbes (Otto and Simpson, 2007).

For several reasons, it is notable that lipid type, lipid origin, and tree species did not have larger effects on lipid concentrations in soil. First, much organic matter in soils is thought to be stabilized by interactions with mineral and organic soil constituents that should depend on the chemical functional groups present on lipids (Kleber et al., 2007). For example, negatively charged carboxylic acid groups could facilitate lipid preservation through interactions with

positively charged surfaces on pedogenic oxides and with metal cations in soil solution or on clay minerals. Second, several authors have suggested that biomolecules in roots should be preferentially stabilized due to the proximity of roots to mineral soil constituents and the stimulation of soil aggregate formation by roots (Rasse et al., 2005; Schmidt et al., 2012). Finally, the small effect of tree species is remarkable because these species have widely divergent effects on soil properties that could influence lipid preservation in soils: among species, soil pH varied from ca. 4 to 5 (Reich et al., 2005), soil calcium and aluminum ions varied as much as 10-fold (Mueller et al., 2012a; Reich et al., 2005), litter decomposition rates and turnover times of the organic horizon varied more than 2-fold (Hobbie et al., 2010; Hobbie et al., 2006), and the abundance of soil decomposers, including bacteria, fungi, and anecic earthworms, was also highly variable (Hobbie et al., 2007; Reich et al., 2005). Notably, the effects of lipid type, chain length, and lipid origin were also similar for all tree species ($P > 0.3$ for interactions of these factors with species identity).

To estimate the sensitivity of soil lipid concentrations to changes in different predictor variables, we compared least-squares adjusted means and standardized model coefficients from the model with all factors included. The adjusted means of ω -hydroxyacids, dihydroxyacids, and α,ω -diacids were ca. 30% more abundant than the mean soil lipid concentration, while n -alcohols, n -alkanoic acids, and trihydroxyacids were ca. 25% less abundant than the mean soil lipid concentration. Long-chain lipids were more abundant in soil than expected according to the other factors in the model; our model predicted that increasing chain length from 15 to 22 carbon atoms would increase soil lipid abundance by 37% (Table 2). Additional studies are needed to determine if similar

Table 2
Estimates of variability for each predictor of soil lipid concentrations and predicted effects on soil lipid concentrations associated with changes in the value of each predictor.

Predictor	Range	Lower quartile	Upper quartile	Increase in soil lipid concentration by shifting from the lower to the upper quartile (%) ^a	Increase in soil lipid concentration by shifting from the min. to the max. value (%) ^a
Lipid concentration in leaves and roots of <i>present</i> plantations ($\mu\text{g g}^{-1}\text{C}$)	20–23,077	310	2095	91	897
Lipid concentration in leaves and roots of <i>previous</i> plantation ($\mu\text{g g}^{-1}\text{C}$)	32–12,350	210	1532	96	647
Lipid carbon chain length (number of carbon atoms)	8–30	15	22	37	168
Proportion root-derived (=root conc./leaf + root conc.)	0–1	0.22	0.77	16	31

^a Predicted increases in soil lipid concentrations are based on the estimates of variability, the average of log-transformed soil lipid concentrations (6.16, equal to 472 $\mu\text{g g}^{-1}\text{C}$), and the model coefficients for each predictor from a model that included each predictor given in the table and two others: lipid type and tree species identity. The predicted increases due to shifting from the minimum predictor value to the maximum reflect the extent of *potential* effects of a given predictor on soil lipid concentrations. In contrast, the predicted changes using the quartile values are a type of standardized model coefficient that normalizes for differences in the variability of each predictor (Bring, 1994).

patterns exist in other soils and whether these patterns are due to: (i) inherent lipid properties, such as lower water solubility of long-chain lipids or hydrophobic interactions between long-chain lipids, (ii) the association of lipids with different plant biopolymers or undocumented sources, e.g. bark, or (iii) differences in the location of lipids within plant tissues and biopolymers. The lower adjusted-mean concentrations of *n*-alcohols could result from preferential degradation caused by their terminal position in plant polyesters (Graça and Santos, 2007). The higher adjusted-mean concentrations of ω -hydroxyacids, dihydroxyacids, and diacids cannot strictly be due to their presence in cutin and suberin because other monomers of cutin and suberin, specifically the trihydroxyacids, had low adjusted-mean concentrations. Bark, which contains suberin and thus is a potential source of the hydroxyacids and diacids in soils, probably does not account for the differences among lipid types because woody litter accounted for <20% of total litterfall in all plantations (Oleksyn unpublished) and bark would be a very small fraction of woody litterfall.

According to standardized model coefficients, lipids present exclusively in tree roots were predicted to be 31% more abundant in mineral soils than lipids present exclusively in leaves (Table 2). These results suggest there is a *generic* mechanism that causes root-derived lipids to be more abundant in mineral soils than leaf-derived lipids (Mueller et al., 2012b; Nierop, 1998; Otto and Simpson, 2006; Rumpel et al., 2002); i.e., the relative abundance of root-derived lipids in soil is partly independent of differences between chemical properties of root and leaf-derived lipids (Fig. 1) and variation in soil properties among plantations of different species (Table 1), including the abundance of anecic earthworms and the abundance and decomposition of leaf and root litter (Hobbie et al., 2010; 2006; Reich et al., 2005; Withington et al., 2006).

Here, we have provided the first quantitative estimates of the effect of plant lipid properties on their concentrations in soil. The concentrations of *n*-alkanoic acids, α,ω -diacids, *n*-alcohols, and hydroxyacids in soil were very strongly correlated with their concentrations in leaves and roots, with much smaller effects of lipid biochemical properties, lipid origin, and tree species identity (which had large effects on biogeochemical cycling and biota in soil). Additional studies should evaluate, using similar quantitative methods, whether other molecular constituents of soil organic matter are similarly influenced by their biochemical properties and origins.

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