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Dynamics of microbial communities during decomposition of litter from pioneering plants in initial soil ecosystems

J. Esperschütz[1,2], C. Zimmermann[3], A. Dümig[4], G. Welzl[2], F. Buegger[5], M. Elmer[6], J. C. Munch[1,5], and M. Schloter[2]

[1]Technische Universität München – Chair of Soil Ecology, Center of Life and Food Sciences Weihenstephan, Ingolstaedter Landstr. 1, 85764 Neuherberg, Germany
[2]Helmholtz Zentrum München, GmbH German Research Center for Environmental Health, Research Unit for Environmental Genomics, Ingolstaedter Landstr. 1, 85764 Neuherberg, Germany
[3]Brandenburg University of Technology – Chair of Soil Protection and Recultivation, P.O. Box 101344, 03013 Cottbus, Germany
[4]Technische Universität München – Chair of Soil Science, Center of Life and Food Sciences Weihenstephan, Emil-Ramann-Straße, 85354 Freising, Germany
[5]Helmholtz Zentrum München GmbH, German Research Center for Environmental Health, Institute of Soil Ecology, Ingolstaedter Landstr. 1, 85764 Neuherberg, Germany

[6]Brandenburg University of Technology, Research Centre Landscape Development and Mining Landscapes, Konrad-Wachsmann-Allee 6, 03046 Cottbus, Germany

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Correspondence to: M. Schloter (schloter@helmholtz-muenchen.de)

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Abstract

In initial ecosystems concentrations of all macro- and micronutrients can be considered as extremely low. Plant litter therefore strongly influences the development of a degraders' food web and is an important source for C and N input into soil in such ecosystems. In the present study, a ^{13}C litter decomposition field experiment was performed for 30 weeks in initial soils from a post-mining area near the city of Cottbus (Germany). Two of this regions' dominant but contrasting pioneering plant species (*Lotus corniculatus* L. and *Calamagrostis epigejos* L.) were chosen to investigate the effects of litter quality on the litter decomposing microbial food web in initially nutrient-poor substrates. The results clearly indicate the importance of litter quality, mainly the amount of N stored in the litter material and its bioavailability for the degradation process and the development of microbial communities in the detritusphere and bulk soil. Whereas the degradation process of the *L. corniculatus* litter which had a low C/N ratio was fast and most pronounced changes in the microbial community structure were observed 1–4 weeks after litter addition, the degradation of the *C. epigejos* litter material was slow and microbial community changes mainly occurred at between 4 and 30 weeks after litter addition to the soil. However for both litter materials a clear indication for the importance of fungi for the degradation process was observed both on the abundance level as well as on the level of ^{13}C incorporation (activity).

1 Introduction

Whereas initial terrestrial ecosystems are characterized by a dominance of geological processes like rock weathering, biological processes become more and more important during ecosystem development (Gerwin et al., 2009). In this respect the role of pioneering plants colonizing initial substrates are of highly important as they enhance carbon input into soil and influence the development of soil microbial communities. They drive the initial development of soil properties and food webs, mainly through root

importance

morphology, rhizodeposition and litter production (Bardgett et al., 1999; Bardgett and Walker, 2004; Hättenschwiler et al., 2005). As the overall status of nutrients like ammonia and nitrate in initial ecosystems is low, plants benefit in turn from the microbial activities in soil, which act as catalysts of nutrient recycling from dead biomass and for the new input of nutrients (e.g. by nitrogen fixation) (Wardle et al., 2004).

In contrast to the degradation of root exudates, which has been considered as a very fast process, mainly driven by root associated microbes (Walker et al., 2003; Baudoin et al., 2003), that are more related to the plant species than to soil type, many authors have described two phases with highly differing kinetics during litter degradation (Dilly et al., 2003; Fioretto et al., 2005). Whereas in the first phase easily degradable compounds derived from the litter material are fastly transformed mainly by epiphytic microbes, the second phase dedicated to the degradation of substances such as lignin or cellulose is slow and mainly driven by microbes from soil, that colonize the remaining litter material and form a network of complex interactions that are needed to catalyze the corresponding reactions (Aneja et al., 2006). Therefore it has been postulated that litter degradation is more depending on the structure and activity of soil microbes and therefore linked to the evolution and pedogenesis of soils (Van Veen and Kuikman, 1990). Due to the importance of litter degradation for nutrient cycling in soil many studies have been carried out in recent years to investigate the mobilization of carbon and other nutrients in different soil ecosystems, including forest stands (Moore-Kucera and Dick, 2008), agricultural fields (Elfstrand et al., 2008) or tropical soils (Kurzatokowski et al., 2004). Generally in many cases degradation rates of litter were closely linked with the soil nitrogen content, confirming the hypothesis of Frey et al. (2003), who have postulated a reciprocal transfer of carbon and nitrogen at the soil litter interface.

However during the initial phase of soil ecosystem development both parameters, that have been considerd as drivers for litter degradation in soil (abundance of cellulose and lignin degraders as well as nitrogen content) differ in comparison to well developed soils. Therefore to improve our understanding on litter degradation in initial soil ecosystems and to identify the underlying microbial network structures, we performed litter

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degradation studies using ^{13}C-labelled litter from two contrasting plants (*Lotus corniculatus* L. and *Calamagrostis epigejos* L.), which are expected to show differences in litter quality and have been dominantly detected in post-mining areas (Pawlowska et al., 1997; Süß et al., 2004; Gerwin et al., 2009). The experiment was carried out on an experimental area closed to an artificial catchment ("Chicken Creek"), located in an opencast mining area (Gerwin et al., 2009), where initial ecosystem processes as well as ecosystem development are studied. To describe microbial communities involved in litter degradation and initial food web development the ^{13}C contents of phospholipid fatty acids (PLFA) extracted from the soil were measured. We postulated that due to the initial nutrient-poor substrate that was associated with a low abundance litter decomposers, the amount of N derived from plant litter highly influences the performance of the litter degrading microbial biomass resulting in a much faster colonization and degradation of the litter derived from *L. corniculatus*.

2 Materials and methods

2.1 Plant litter labelling

To obtain labelled plant litter, 2 g of *Lotus corniculatus* and 0.3 g of *Calamagrostis epigejos* seeds (BSV Saaten, Germany) were incubated in plastic pans (12 × 55 × 35 cm) using a mixture of potting soil, containing clay and silica sand (2 : 1 : 1, *v*/*v*/*v*) in a greenhouse experiment. Plants were grown at temperatures of +20/+15 °C (day/night) and a relative humidity of between 75 and 85 %. Irrigation was performed daily via irrigation tubes (500 ml/24 h). Biweekly, 500 ml of Hoagland-based fertilizer (25 % strength) was added to each pan after irrigation (Hoagland, 1920). Three weeks after sowing, the pans were transferred into a tent (volume ∼ 7000 l) based on an airtight transparent plastic foil. Air recirculation was achieved using six fans, which were located in the tent corners and in the middle of the longitudinal sides of the tent. The CO_2 in the tent's atmosphere was subsequently replaced with low enriched $^{13}CO_2$ ($\delta^{13}C + 170$ ‰

vs. Vienna-Pee Dee Belemnite (V-PDB), Air Liquide, Düsseldorf, Germany), and the CO_2 within the tent was maintained at 350 and 400 µmol mol^{-1} (monitored by a photoacoustic CO_2-controller, calibration at 300 to 600 µmol × mol^{-1} ± 2 %). Measurement of the ^{13}C atmosphere (three times a day) was conducted via GC-IRMS analyses (Finnigan MAT 253, Bremen, Germany). Using this experimental setup, an enriched ^{13}C-atmosphere of +140 ‰ V-PDB was established during plant growth. The labelled plants were harvested before flowering after a total growth time of six to eight weeks. Above-ground plant parts (stems and leaves) were oven-dried (60 °C) shredded (0.5–2 cm) and homogenised. The labelled plant litter of *C. epigejos* ($\delta^{13}C = 136.8 \pm 0.6$ ‰ vs. V-PDB) and *L. corniculatus* ($\delta^{13}C = 101.3 \pm 2.1$ ‰ vs. VPDB) was used for the subsequent ^{13}C-litter decomposition field study.

2.2 Research site description

The experiment was carried out in the post mining area "Welzow-Süd" (51°37′6″ N/ 14°19′32″ E) close to the observation site "chicken creek" (Lausatia, Germany), where site observation started in September 2005 as recently described by Gerwin et al. (2009). The area is described as an area with temperate climate with sub-continental character, a mean annual temperature of 8.9 °C and comparatively low precipitation (563 mm per year). Soil was characterised as sands to loamy sands. Major soil characteristics can be found in table 1. Seed bank analyses showed only minor quantities of plant seeds, resulting in a low plant density of approximately 0.15 individuals per m^2. Further meteorological data during the experimental period were shown in Fig. S1 (Supplement).

2.3 Experimental setup

In the present experiment the soil microbial community structure and selected soil parameters were investigated at several time points after the application of labelled plant litter material of *L. corniculatus* and *C. epigejos*. Water extractable organic carbon

(WEOC) and microbial community structure based on its phospholipid fatty acids (PLFA) were investigated 1 week, 2 weeks, 4 weeks, 15 weeks and 30 weeks after the application of labelled plant litter in the detritusphere. The ^{13}C labelling was used to trace the C applied with the plant litter into the respective soil and microbial fractions. In addition, litter bags were incorporated into separate tubes to measure the litter decomposition rates during the experimental period.

At the end of April 2009, tubes made of aluminium (20 cm in diameter and 14 cm high) were installed 10 cm deep into the soil. Every treatment (control treatments without litter application (Co), treatments with litter bags (Lb); treatments with direct litter application to soil (Ls)) was carried out in individual tubes and replicated in five separate field plots of 9 × 9 m (Fig. 1). Every tube was protected against disturbances with steel grids on the top (mesh size 5 mm). In Ls tubes plant litter (5 g oven-dried) was mixed directly within the first 5 cm of the upper soil fraction. At every harvesting time point, the detritusphere was harvested and sieved immediately (<2 mm) to remove litter residues from the soil substrate. An aliquot of around 50 g was immediately frozen at −20 °C for PLFA analyses, whereas the remaining amount was stored at 4°C for further analyses described below. In addition, nylon litter bags (10 × 10 cm; mesh size 40 µm) were filled with *L. corniculatus* respectively *C. epigejos* plant litter (5 g oven-dried), and placed in separate tubes within the first 5 cm. At every harvesting time point, one litter bag per plant litter treatment was removed to estimate litter decomposition rates.

2.4 Soil parameters

For analyses of total carbon (TC), total organic carbon (TOC), $\delta^{13}C$ of TC and TOC as well as total nitrogen (TN), dried soil aliquots (60 °C, 72 h) were ball-milled and subsequently weighed into tin capsules (approx. 70 mg) for elemental analysis and mass spectrometry. Inorganic C was estimated using a second set of samples in silver capsules, treated with HCl (30 µl, 32 %) with subsequent evaporation with a vacuum concentrator (Univapo 100 ECH, Montreal Biotech, Canada) at 50 °C for 30 min prior to measurement. An additional tin capsule was put to each sample to ensure an

optimal combustion in the elemental analyzer. Samples were measured vs. the international standard USGS 40 (Eurovector, Milan, Italy, coupled with an isotope ratio mass spectrometer, Delta V, Thermo Electron, Bremen, Germany). Soil pH was analysed in 0.01 M $CaCl_2$ extracts (DIN ISO 10390). Prior to microbial and soil analyses, litter residues have been carefully removed. However, depending on the weather conditions and especially the soil moisture (0–5 cm), a small part plant litter material possibly remained in the soil sample prior to extraction. The remaining litter residues in the soil samples have been quantified by light fraction analyses (>20 µm) between 0.1–0.3 % in all samples (data not shown).

2.5 Litter bag analyses

Litter degradation rates were calculated based on the loss of litter material in the litter bags during incubation after drying at 60 °C. The dried plant litter material was ball-milled and subsequently analysed for TOC, $\delta^{13}C$ and TN content using an elemental analyzer (Eurovector, Milan, Italy) coupled with an isotope ratio mass spectrometer (MAT 253, Thermo Electron, Bremen, Germany).

2.6 WEOC and litter derived ^{13}C in WEOC

Aliquots of 5 g were taken in triplicates for the extraction of WEOC using 20 ml of 0.01 M $CaCl_2$. Samples were shaken in a rotary shaker for 45 min and subsequently filtered through folded filters (595 1/2, Whatman GmbH, Germany) The extracts were stored at −20 °C until measurement. After acidification (2N HCl, 30 µl), measurement of the total organic C (TOC) and $\delta^{13}C$ in the $CaCl_2$ extracts was done by on-line coupling of liquid chromatography and stable isotope ratio mass spectrometry (LC-IRMS, Thermo Electron, Bremen, Germany) according to Krummen et al. (2004) and Marx et al. (2007).

2.7 PLFA analyses

Phospholipid fatty acid (PLFA) analyses were performed based on Zelles et al. (1995). An aliquot of 50 g soil (dry weight) was extracted with 250 ml of methanol, 125 ml of chloroform and 50 ml of phosphate buffer (0.05 M, pH 7). After 2 h of horizontal shaking, 125 ml of water and 125 ml of chloroform were added to promote phase separation. After 24 h the water phase was removed and discarded. The total lipid extract was separated into neutral lipids, glycolipids and phospholipids on a silica-bonded phase column (SPE-SI 2 g/12 ml; Bond Elut, Analytical Chem International, CA, USA). The phospholipid extract was further separated into saturated (SATFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids (see Zelles et al., 1995 for details) to facilitate the identification of fatty acids as well as to obtain a good baseline separation of peaks for isotopic calculations.

Prior to measurements, internal standards (nonadecanoic acid methyl ester respectively myristic acid methyl ester) were added to calculate absolute amounts of fatty acids. PLFA were analyzed as fatty acid methyl esters (FAME) on a gas chromatograph/mass spectrometry system (5973MSD GC/MS Agilent Technologies, Palo Alto, USA). FAMEs were separated on a polar column (BPX-70, SGE GmbH, Griesheim, Germany), 60 m × 0.25 mm × 0.25 µm, coated with 70 % of cyanopropyl polysilphenylene-siloxane (see Esperschütz et al., 2009 for details). The mass spectra of the individual FAME were identified by comparison with established fatty acid libraries (Solvit, CH 6500-Luzern, Switzerland) using MSD Chemstation (Version D.02.00.237). The ^{13}C signature of the corresponding PLFA was determined by online coupling of the GC/MS system with an isotope ratio mass spectrometer (IRMS, Delta Advantage, Thermo Electron cooperation, Bremen, Germany) after combustion (GC Combustion III, Thermo Electron Cooperation, Bremen, Germany). The actual $\delta^{13}C$ ratio of the individual FAME was corrected for the one C atom that was added during derivatisation (Abrajano et al., 1994). Fatty acids are presented by the number of C atoms followed by the number of double bonds. The positions of double bonds are indicated by "ω"

and the number of the first double-bonded C atoms from the ω end of the C chain. Anteiso and iso-branched fatty acids are indicated by "a" and "i", followed by the number of C atoms. Branched fatty acids in which the position of the double bond was unknown, were indicated by the prefix "br". Methyl groups on the tenth C atom from the carboxyl end of the molecule were indicated by "10ME". Cyclopropane fatty acids were indicated by the prefix "cy", while dicyclopropylic PLFA were indicated by "dic". Even-chained, saturated fatty acids were abbreviated with the prefix "n".

2.8 Calculations

Stable isotope results were expressed in $\delta^{13}C$ or atom percent (AP) according to Eqs. (1) and (2):

$$\delta^{13}C = [(R_{sample} / R_{V\text{-}PDB}) - 1] \times 1000 \quad (1)$$

$$^{13}C_{AP} = \frac{100 \times R_{V\text{-}PDB} \times (\delta^{13}C / 1000 + 1)}{1 + R_{V\text{-}PDB} \times (\delta^{13}C / 1000 + 1)} \quad (2)$$

R_{Sample} and $R_{V\text{-}PDB}$ represent the ^{13}C to ^{12}C ratios of sample and international standard Vienna-Pee Dee Belemnite (V-PDB = 0.0111802), respectively. The relative amount of litter-incorporated ^{13}C ($\%^{13}C_{LITTER}$) into the total lipid fraction or into the amount of WEOC was calculated according to Eq. (3),

$$\%\,^{13}C_{LITTER} = \frac{C_{Tx} \times (^{13}C_{Tx} - {}^{13}C_{T0}) \times 100}{^{13}C_{added}} \quad (3)$$

where the concentration of the individual C-fraction [ng g^{-1} DW] at time point Tx was multiplied by its ^{13}C enrichment in atom percent excess (difference between ^{13}C at time point Tx and ^{13}C enrichment at control) and expressed relatively to the amount

of added ^{13}C [ng g^{-1} DW]. The relative ^{13}C-distribution within total measured phospholipids (% $^{13}C_{DIST}$) was calculated as follows:

$$\%\,^{13}C_{DIST} = \frac{\%\,^{13}C_{LITTER}i \times 100}{\sigma\,\%\,^{13}C_{LITTER}i} \quad (4)$$

where % $^{13}C_{LITTER}i$ represents the relative amount of added ^{13}C in an individual phospholipid i, as calculated after Eq. (4).

2.9 Statistics

An univariate analysis of variance was carried out using SPSS 11.0 (SPSS Inc.), with harvesting time (0 weeks, 1 week, 2 weeks, 4 weeks, 15 weeks and 30 weeks) and litter treatment (control, *L. corniculatus* and *C. epigejos*) used as independent variables. A two-way analysis of variance (ANOVA) was performed to establish significant interactions between the harvesting time and treatment. Significant differences for specific variables were identified using a Duncan's post-hoc test at $p < 0.05$ following a one-way ANOVA. Exponential curve-fitting (Fig. 2) was performed with Sigma Plot 11.2 (Systat software Inc.). Data illustration was performed with Adobe Illustrator CS3 and S-PLUS 8.1 (Tibco Software Inc.). Results are presented as means ($n = 5$) with standard deviations given in brackets.

Soil microbial community profiles were analyzed using principal response curves (PRC). This method was originally suggested to study the effect of different treatments on ecological communities with repeated observations in time (van den Brink and ter Braak, 1999). PRC can be interpreted as special case of Redundancy Analysis (function prc in library vegan, R-project). It has been shown (Schramm et al., 2007) that PRC is equivalent to a two-step procedure, which involves the transformation of data (centring with respect to time and averaging according to treatment groups) and a principal component analysis with the transformed data. Using the R environment for statistical

computing http://www.R-project.org this algorithm was realized and used to generate Figs. 4, 5, 6.

3 Results

3.1 Plant litter degradation

For both plant litter types significant degradation rates were observed during the experimental period of 30-week (Fig. 2) with faster degradation rates for *L. corniculatus* over the whole experimental period. At the final sampling 50 % of the applied litter material from *C. epigejos* and 30 % from *L. corniculatus* remained undegraded degraded. For both litter types initial degradation rates in the first 10 weeks after application were higher compared to the degradation rates observed afer 30 weeks of incubation. The N content of *L corniculatus* was significantly higher compared to *C. epigejos* plant litter, which resulted in a C/N ratio of around 40 for *C. epigejos* compared to 15 for *L. corniculatus*. Whereas the C/N ratio did not change over the experimental period in the litter from *L. corniculatus* the C/N of *C. epigejos* litter decreased; at the last sampling time point here a C/N ratio of 25 was measured.

3.2 WEOC, microbial biomass and litter derived ^{13}C

The WEOC content in soil significantly increased independent from the litter type already one week after litter application compared to the control soil (Fig. 3) indicating a fast release of easy degradable substances from the litter material. At that time point in the *L. corniculatus* treatments a 2.5 fold higher WEOC content was detected compared to the soil samples which were treated with *C. epigejos* litter (250 mg kg^{-1} dw compared to 100 mg kg^{-1} dw). This high amount of WEOC decreased sharply in both treatments and was comparable to the control treatments after 15 weeks of litter incubation independent from the plant litter type. Incorporation of ^{13}C followed the same trend and

confirmed that the increase of WEOC in the first weeks of incubation originated from the litter material.

Soil microbial biomass was estimated on the basis of total PLFA. The application of *L. corniculatus* plant litter stimulated total microbial biomass during the first four weeks of the experimental period with maximum values one week after litter application (Fig. 3b). In contrast, no significant increase was observed in the *C. epigejos* treatments. Only 15 weeks after litter application, the microbial biomass in *C. epigejos* was slightly increased, compared to the control treatments. Again, similar results were observed following the ^{13}C signature over time.

3.3 Soil microbial community profile and ^{13}C incorporation

The litter type had a pronounced influence on the structure of microbial litter degraders as indicated by principal response curves (PRC) of both litter types, based on individual PLFA mol % indicated that. In the *L. corniculatus* treatment, component 1 and 2 accounted for 61.6 % and 35.1 % of the total variance 1 week after litter application (Fig. 4). At that time point higher proportions of the fatty acid 18 : 3 were detected compared to the control treatments without litter application, whereas a decrease occurred mainly related to cy19 : 0, 16 : 1ω7, 18 : 1ω9 and 18 : 1ω7. In addition high loadings of PLFA 18 : 2ω6,9, n20 : 0, a15 : 0, 18 : 2ω6,9 and n24 : 0 resulted in a separation of the controls from the *L. corniculatus* treatments at the early sampling time points. In contrast differences were low compared to the control treatments at the last sampling time points 15 and 30 weeks after litter application.

In *C. epigejos* treatments, differences in microbial community structure at the early sampling time points were low and developed over time. Main differences were found 4 weeks and 30 weeks after litter application. At that time points component 1 and 2 accounted for 69.1 % (component 1; 4 weeks) and 20.3 % (component 2; 30 weeks) of the total variance (Fig. 5). Most pronounced was a large increase of PLFA 18 : 2ω6,9 four weeks after litter application compared to the control samples; also higher proportions of a15 : 0 were observed, however to a less extent. During this period, n16 : 0,

br18 : 0 and a15 : 0 were reduced compared to the control soils. At the end of the experiment after 30 weeks the PLFA profile was dominated by 18 : 2ω6,9, 18 : 3, 18 : 1ω7 and 16 : 1ω7.

The ^{13}C signature of the corresponding PLFA was used to identify the active part of the microbial community involved in the litter degradation. *L. corniculatus* and *C. epigejos* treatments were compared in a PRC based on the percentage distribution of litter derived ^{13}C among individual PLFA. The results clearly indicate significant differences in response to the litter type and time points under investigation. Overall component 1 and 2 could explain 75.2 % and 21 % of the total variance (Fig. 6). At the early time points of sampling, plant litter derived ^{13}C was mainly found in PLFA 18 : 3 and to a lower extend in PLFA 18 : 2ω6,9 and a15 : 0 in the *L. corniculatus* treatments. This increase was still visible at the end of the experiment. In contrast in soil samples, where *C. epigejos* litter has been applied, a major part of the plant litter derived carbon could be detected in PLFA 18 : 2ω6,9 one and two weeks after litter application, whereas at later sampling time points high amounts of ^{13}C label were measured in PLFA 15 : 0, n16 : 0 and i15 : 0.

4 Discussion

4.1 Plant litter degradation

During the experimental period of 30 weeks, the amounts of the applied plant litter of *L. corniculatus* as well as *C. epigejos* decreased significantly. However the total loss of litter mass was lower in the field study than in a comparable microcosm experiment (Esperschütz et al., 2011), which indicates the importance of climatic conditions for the degradation of plant derived litter material. Whereas in the microcosm study optimal conditions were chosen for litter degradation (including soil water content and temperature), these parameters are highly dynamic in time under field conditions. Mainly at the end of the incubation period (starting 21 weeks after addition of the plant litter

material) temperature and soil moisture dropped significantly and may have influenced the degradation of the plant litter material in this phase (see Fig. S1, Supplement). Decomposition rates of litter mainly depend on the ratio of easily degradable substances to more recalcitrant compounds respectively substances with antimicrobial properties (Berg et al., 2000; Palosuo et al., 2005). The N content of *C. epigejos* plant litter was initially lower in (1.08 ± 0.03) but similar in its C content (43.75 ± 0.22) compared to the *L. corniculatus* plant litter (3.02 ± 0.12 respectively 42.97 ± 0.76). The high C/N ratio of *C. epigejos* obviously results in (40.46 ± 1.14) and hence lower attractivity for microbial degraders mainly as ammonia and nitrate concentrations in the soil samples were low or below the detection limit (data not shown), which confirms our initial hypothesis. Consequently during the first four weeks of incubation the mass loss of *L. corniculatus* plant litter might be linked to large amounts of water soluble plant litter components, rich in nitrogen content. Those compounds could be used by microbes colonising the litter material to increase their activity and biomass (Aneja et al., 2006; Poll et al., 2008), confirming previous data from Hopkins et al. (2007). In their study they postulated a close link between decomposition rates of plant litter and nutritional quality in volcanic soils with a comparable nutrient status like at the initial sites of "Chicken Creek".

4.2 WEOC and total soil microbial biomass

An increase of WEOC in soil was detected in all treatments, where litter had been applied already one week after application (Fig. 3a). The parallel increase in ^{13}C in the WEOC indicated that this increase can be directly linked to the applied litter material (Fig. 3c). These results suggest a fast translocation of easy available organic C into the WEOC fraction. Rainfall after one week of litter incubation (Fig. S1) might have increased the transformation of plant litter derived C compounds into the WEOC fraction of soil, since plant residues may contain up to 25 % of water-soluble materials (Swift et al., 1979). After two weeks, the high WEOC content in the *L. corniculatus* treatments decreased and no statistical significant differences were detected between *L. corniculatus* and *C. epigejos*, neither in the absolute content (Fig. 3a) nor the litter derived

^{13}C proportion (Fig. 3c). This might be explained by a higher microbial biomass and activity in the *L. corniculatus* treatments (Fig. 3b, d), which in cooperated most of the easy degradable plant litter derived carbon.

In addition plant litter derived carbon from *C. epigejos* may be more recalcitrant compared to *L. corniculatus* plant litter and hence a less attractive C source for microbes, since only a small fraction of the soil microbial community is likely to possess enzymes necessary to degrade the residue-derived compounds (Williams et al., 2006). Whereas in *L. corniculatus* treatments, microbial litter decomposition was highest within the first 4 weeks after litter application, in *C. epigejos* treatments, litter degrading microbes seem to be more active between week 4 and 15 (Fig. 3b, d). Recalcitrant C compounds of *C. epigejos* might have favoured the development of a more specific microbial community, which is able to utilize complex C substrates. At 15 weeks after litter application, no differences in WEOC and microbial biomass or its corresponding ^{13}C signatures could be detected between the control treatment and both litter treatments; hence easy available C sources have been translocated within the soil matrix.

4.3 Soil microbial community profile and ^{13}C incorporation

According to the PRC analyses (Fig. 4), high proportions of 18 : 3 polyunsaturated fatty acids were dominating the *L. corniculatus* treatment immediately after litter application, which indicates high proportions of eukaryotes at this stage of litter degradation being involved in the transformation (Zelles et al., 1995; Ruess et al., 2007). This is also confirmed by the high ^{13}C signature in the corresponding fatty acid (Fig. 6). As the amount of litter material that has not been removed before lipid extraction was <0.3 %, the amount of 18 : 3 derived from plant litter material is negligible. Therefore we postulated that these fatty acids are linked to microeukaryotes (Zelles et al., 1995; Ruess and Chamberlain, 2010), which may be responsible for breaking small litter fragments and hence preparing the plant litter structure for microbial uptake. PLFA 18 : 2ω6,9 and a15 : 0 which were also increased in abundance and in their specific ^{13}C label,

illustrated a high contribution of soil fungi and Gram-positive bacteria mainly between two and four weeks after litter application in the degradation, which confirms studies by Poll et al. (2008) and Esperschütz et al. (2011). A limited soil N content in the substrate may have stimulated fungi to use plant derived nitrogen (Fig. 4), as suggested earlier in an experiment using a similar substrate (Esperschütz et al., 2011). After 15 weeks of litter incubation, the microbial community structure detected in the *L. corniculatus* litter treatment was similar to the control treatments without litter addition, indicating that most of the introduced plant-derived carbon litter has already been utilized by the microbial biomass.

Comparable to *L. corniculatus* treatments, also in treatments with *C. epigejos*, fungi (18 : 2ω6,9) and Gram-positive bacteria (a15 : 0) benefit from the new plant litter derived C and N; however the increase in abundance was delayed compared to the treatments where *L. corniculatus* was applied and only visible first 4 weeks after litter application (Fig. 5), which might be related to the low availibility of N and other nutrients in soil. Again the increase in the ^{13}C signature in the corresponding fatty acids confirmed the role of these microbes in the plant litter decomposition process (Fig. 6). Obviously only small amounts of easy available C were provided by the *C. epigejos* plant litter, hence Gram-negative bacteria decreased over time. After 30 weeks of incubation an increase of 18 : 2ω6,9 and a15 : 0 on PC1 and 18 : 2ω6,9, 18 : 3, 16 : 1ω7 and 18 : 1ω7 on PC2 indicated a microbial decomposer community which is able to degrade plant litter compounds, which can be considered as more recalcitrant (Kuzyakov et al., 2000; Rubino et al., 2010) for *C. epigejos,* which was not present in *L. corniculatus*. It seems like in *L. corniculatus* treatments the microbial community adapted to the high amounts of easy available C and N sources. In contrast to that, the recalcitrant plant litter favoured the development of a complex and sustainable microbial community structure capable to utilize diverse C sources, even if easy available C compounds have been readily degraded.

Mainly in the treatments with *C. epigejos* litter fungi seem to play an important role in the initial phase of litter degradation in poor substrates, which confirms results from

a previous experiment (Esperschütz et al., 2011). The high ^{13}C content within the fungal biomass after two weeks of incubation (Fig. 6) might be a result of fungal hyphae which were grown from the mineral soil layer into the litter (Moore-Kucera and Dick, 2008) and subsequently provide nutrient sources for other organisms. However recalcitrant N components may have stimulated fungi and at the end of the experiment may have outcompeted Gram-positive bacteria. Both groups of microbes have been described also in other studies in connection with the degradation of complex substrates (Kuzyakov et al., 2000; Dilly et al., 2004; de Boer et al., 2005; Rubino et al., 2010).

5 Conclusions

(1) Independent from the litter quality the importance of fungi for the degradation process was clearly visible based on the abundance data of PLFA 18 : 2ω6,9 as well as specific ^{13}C incoperation rates into this marker. This confirms data from other studies in well developed soil ecosystems, where also fungi have been identified as major group of organisms which drive litter degradation (Mc Mahon et al., 2005). However to answer the question if the same species contribute to litter degradation in initial and well developed soil ecosystems further molecular studies are needed, as the resolution of PLFA analysis is too narrow for further differentation. (2) The kinetics of colonization and the subsequent activity of the microbial communities are strongly linked to the availability of N. Whereas in well developed ecosystems the amount of N needed is provided mainly by the soil (Frey et al., 2003), in initial ecosystems N contents in soil are low and N must be provided by the litter material itself. In this study higher C/N content in *C. epigejos* plant litter, corresponding to absolute lower N contents, resulted in lower microbial biomass and hence slower litter degradation rate. However, towards the end of the vegetation period N was provided by recalcitrant N compounds, whereas easy available N compounds of *L. corniculatus* plant litter on the other hand attract microbial decomposers at the beginning. Therefore different pioneering plants sustain the nutritional (N) state of the initially poor substrate. In the case of *L. corniculatus* the

amount of N provided by the litter may result in the creation of nutrient-rich patches beneath the initially poor substrate material. Such nutrient-rich environments may stimulate the colonisation with coexisting plants like *C. epigejos*, as they are known to tolerate nutrient-poor soil conditions for a long time, but can grow fastly under N-rich conditions (Brezina et al., 2006; Tůma et al., 2009). In this context, the response of microbial communities to different mixtures of plant litter material, but also to different pioneering plants' rhizodeposition might be an interesting topic for future research, mainly to transfer this knowledge to the development of practical application for soil restoration.

Supplementary material related to this article is available online at: http://www.biogeosciences-discuss.net/9/14981/2012/bgd-9-14981-2012-supplement.pdf.

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Table 1. Major soil parameters (0–5 cm depth) of the soil substrate (without litter application) throughout the experimental period ($n = 5 \pm$ standard deviation).

	Harvesting time points 2009					
soil parameter 0–5 cm (controls without litter)	29 April 0 weeks	6 May 1 week	13 May 2 weeks	26 May 4 weeks	12 August 15 weeks	30 November 30 weeks
pH	8.4 ± 0.1	8.4 ± 0.1	8.4 ± 0.1	8.5 ± 0.1	8.5 ± 0.0	8.3 ± 0.1
TC [%]	0.42 ± 0.33	0.44 ± 0.29	0.47 ± 0.33	0.47 ± 0.37	0.44 ± 0.31	0.49 ± 0.25
TOC [%]	0.26 ± 0.10	0.25 ± 0.11	0.22 ± 0.08	0.24 ± 0.05	0.30 ± 0.12	0.37 ± 0.14
TN [%]	0.008 ± 0.003	0.009 ± 0.004	0.009 ± 0.002	0.009 ± 0.003	0.010 ± 0.004	0.009 ± 0.004
C_{org}/N	33.1 ± 5.3	29.3 ± 8.1	25.1 ± 3.7	26.6 ± 5.3	31.6 ± 10.3	42.2 ± 13.7
δ^{13}C TOC [‰ vs. V-PDB]	−26.1 ± 0.8	−26.1 ± 0.7	−26.2 ± 0.6	−26.2 ± 0.7	−27.2 ± 0.7	−27.5 ± 1.2

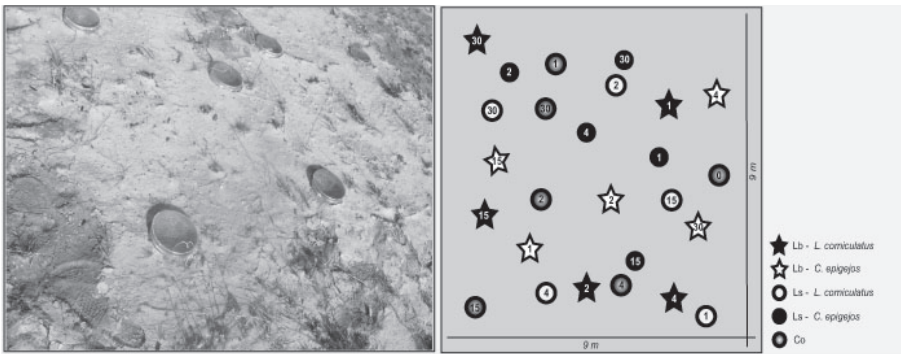

Fig. 1. Design of one field plot (9 × 9 m), replicated 5 times on the research site "Welzow Süd". Detritusphere (Ls) and litter bag treatments (Lb) were harvested 1, 2, 4, 15 and 30 weeks after litter application. Control samples without litter application (Co) were additionally harvested at every time point.

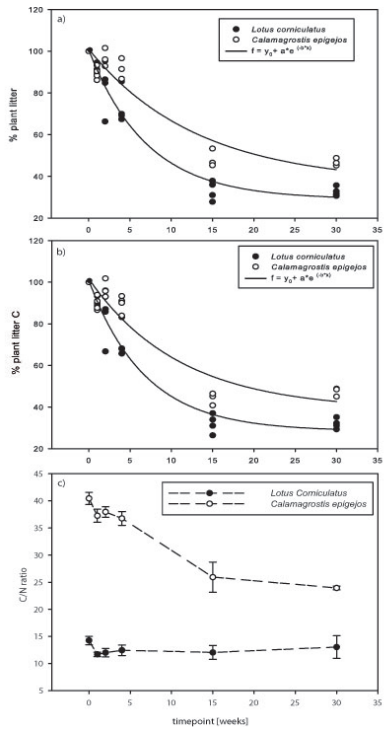

Fig. 2. Total plant litter mass loss **(a)**, C loss **(b)** and C/N ratio **(c)** of the plant litter throughout the experiment. Results are presented as single values **(a, b)** or means ± standard deviation **(c)** based on four replicates.

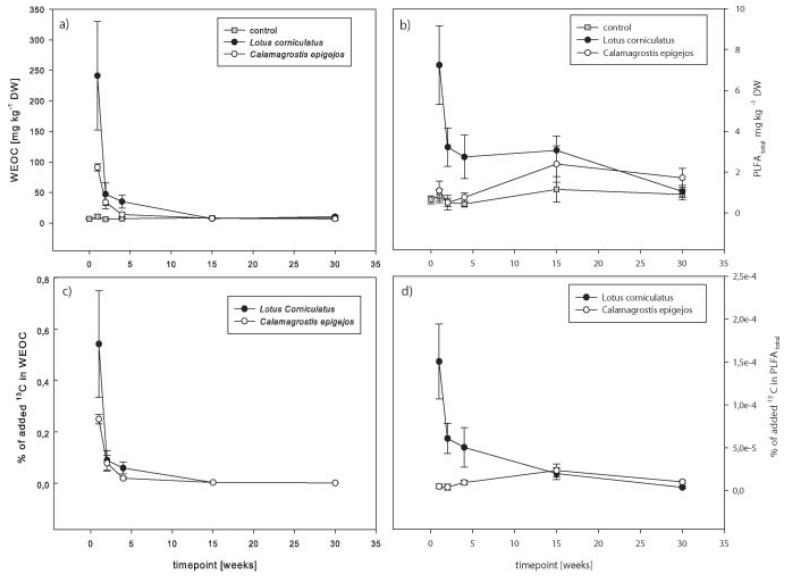

Fig. 3. WEOC **(a)** and soil microbial biomass **(b)** based on and total PLFA in *L. corniculatus* and *C. epigejos* treatments [mg kg^{-1} DW]. Relative amount [%] of litter-derived ^{13}C in WEOC **(c)** and soil microbial biomass **(d)**; values are were expressed as a percentage of initially added ^{13}C, normalised with controls. Results are shown with standard deviation (n = 5).

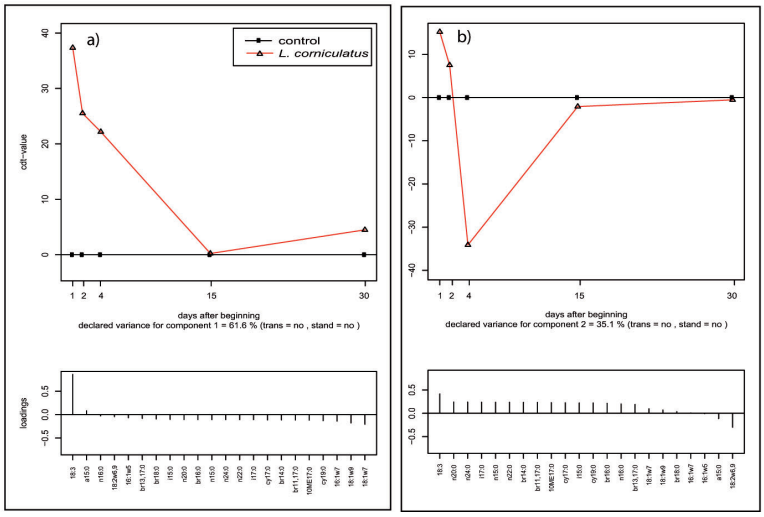

Fig. 4. First **(a)** and second **(b)** component of the PRC calculation on the basis of the mol % data of all individual PLFA relative to total PLFA of *L. corniculatus* treatments compared to the control treatment throughout the experimental period of 30 weeks (n = 5).

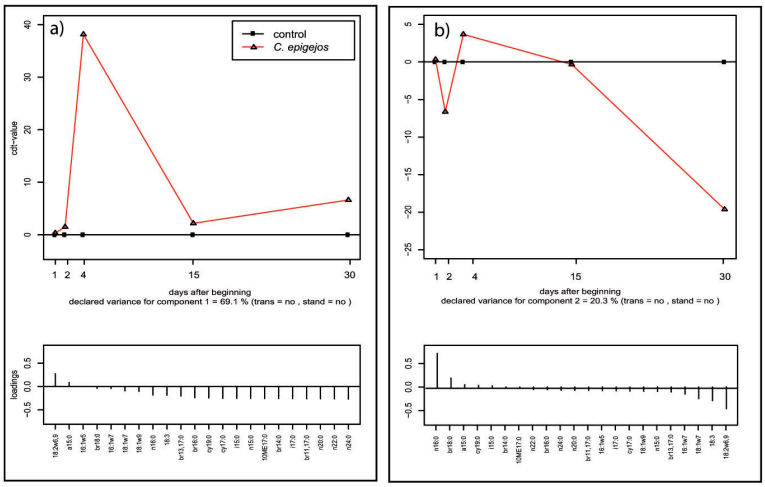

Fig. 5. First **(a)** and second **(b)** component of the PRC calculation on the basis of the mol % data of all individual PLFA relative to total PLFA of *C. epigejos* treatments compared to the control treatment throughout the experimental period of 30 weeks (n = 5).

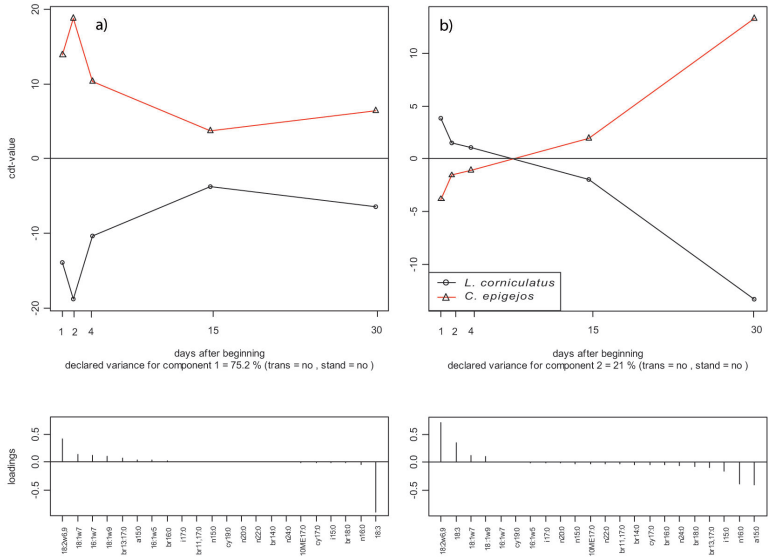

Fig. 6. First **(a)** and second **(b)** component of the PRC calculation on the basis of the percentage distribution of litter ^{13}C within the PLFA composition in *C. epigejos* and *L. corniculatus* throughout the experimental period of 30 weeks (n = 5).