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Litter decomposition in peatlands is promoted by mixed plants

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Abstract

Purpose. The carbon sink function of peatlands is primarily driven by a higher production than decomposition of the litter *Sphagnum* mosses. The observed increase of vascular plants in peatlands could alter the decomposition rate and the carbon (C) cycle through a litter mixing effect, which is still poorly studied. Here, we examine the litter mixing effect of a peat moss (*Sphagnum fallax*) and two vascular plants (*Pinus uncinata* and *Eriophorum vaginatum*) in the field and laboratory-based experiment.

Materials and methods. During the laboratory incubation, mass loss, CO_2 production and dissolved organic carbon concentration were periodically monitored during 51 days. The collected data were then processed in a C dynamics model. The calculated enzymatic activity was correlated to the measured β-glucosidase activity in the litter. In the field experiment, mass loss and CO_2 production from litter bags were annually measured for three years.

Results and discussion. Both laboratory and field experiments clearly show that the litter mixture, i.e. *Sphagnum-Pinus-Eriophorum,* had a synergistic effect on decomposition by enhancing the mass loss. Such enhanced mass loss increased the water extractable C and CO_2 production in the litter mixture during the laboratory experiment. The synergistic effect was mainly controlled by the *Sphagnum-Eriophorum* mixture that significantly enhanced both mass loss and CO_2 production. Although the β-glucosidase activity is often considered as a major driver of decomposition, mixing the litters did not cause any increase of the activity of this exo-enzyme in the laboratory experiment suggesting that other enzymes can play an important role in the observed effect.

Conclusions. Mixing litters of graminoid and *Sphagnum* species led to a synergistic effect on litter decomposition. In a context of vegetation dynamics in response to environmental change, such a mixing effect could alter the C dynamics at a larger scale. Identifying the key mechanisms responsible for the synergistic effect on litter decomposition, with a specific focus on the enzymatic activities, is crucial to better predict the capacity of peatlands to act as C sinks.

Keywords β-glucosidase • Carbon dynamics models • Catalysis • CO_2 production • Dissolved organic carbon • Litter mixture effect

1. Introduction

Sphagnum-dominated peatlands accumulate organic matter (OM) as peat at a rate of ca. 20 to 30 g C m^{-2} $year^{-1}$ (Francez 2000; Rydin et al. 2013). At global scale, peatlands are estimated to store about 270 to 547 Pg C as peat (1 Pg = 10^{15} g), representing ca. 15-30% of the world's soil carbon (C) stock in an area accounting for only 3-5% of the land surface (Turunen et al. 2002; Yu et al. 2010). This high rate of C accumulation is due to peculiar environmental and soil conditions, i.e. waterlogging, anoxia, acidity, low temperature, and specific plant species composition that ultimately hamper the microbial litter decomposition so resulting in a net accumulation of OM as peat (e.g. Gorham, 1991; Holden, 2005). In particular, *Sphagnum* mosses have a key role for peat accumulation as they are able to create unfavorable conditions for decomposer activities by producing a recalcitrant litter and by promoting waterlogged and acidic conditions (Van Breemen 1995). As a result, *Sphagnum* mosses gain in competitive ability against vascular plants, whose litter is much more easily decomposable (Bragazza et al. 2007, 2009), thereby acting as effective ecosystem engineers (Van Breemen 1995).

Human-induced environmental changes are expected to modify plant species abundance in peatlands, in particular by favoring vascular plants at the expense of *Sphagnum* mosses under a warmer climate (Buttler et al., 2015; Dieleman et al., 2015). Although previous studies have shown that warming can modify the rate of litter decomposition in peatlands (Thormann et al., 2004; Bragazza et al. 2016) and that decomposition rates of *Sphagnum* are lower than those of vascular plants (Hoorens et al., 2010), few studies have addressed the issue of a litter mixture effect in peatlands (Hoorens et al. 2010; Krab et al. 2013; Gogo et al. 2016). Most of the studies on litter decomposition in peatlands have focused on single plant species, although in natural conditions litters mainly consist of a mixture of multiple plant species (Salamanca et al. 1998). Litters in mixture can decompose faster (synergistic effect) or slower (antagonistic effect) than the same litter type alone (non-additive effect (Gartner and Cardon, 2004). Almost 70% of mixed-species litter exhibited non-additive mass loss with a prevalence of synergistic effects (Gartner and Cardon, 2004). Such modifications of the decomposition rate due to litter mixture could affect the imbalance between primary production and decomposition in peatlands in response to a vegetation dynamics and, ultimately, the capacity of peatlands to act as C sinks. Despite the crucial role of litter decomposition in controlling C dynamics and peat accumulation in peatlands, we have still little understanding of how the mixture litter affects decomposition.

As change in leaf litter quality can affect enzyme production by soil microbes (Hu et al. 2006), litter mixture could also change enzyme activities, which may result in a non-additive effect. For example, β-glucosidase is commonly described as an important exo-enzyme involved in C-cycling, and therefore primarily in the decomposition of cellulose (Kourtev et al. 2002; Sinsabaugh et al. 2002), so that it could be a key-player in the non-additive effect of litter mixture. Nevertheless, the role of enzymes in relation to litter decomposition still needs to be elucidated.

In order to understand the effect of a litter mixture of *Sphagnum* mosses and vascular plants on OM decomposition and C dynamics in peatlands, we performed a decomposition experiment under laboratory and field conditions. Experimental laboratory data were then used to calibrate a C dynamics model proposed by Gogo et al. (2014), using three C compartments: the solid (mass loss), the dissolved (Water Extractable Organic Carbon, WEOC) and the gaseous components (CO_2-C). In this model, the litter is catalyzed by exo-enzymes at a rate “c” to produce soluble organic C, i.e. WEOC, which is used for respiration at a rate “r” by microorganisms. The model gives a catalysis rate for each litter that contributes to overall catalytic activity. The field experiment was performed to assess whether there was agreement between laboratory and field decomposition tests by focusing on mass loss and CO_2 production. Overall, the aims of this work were: (i) to determine the occurrence of a litter mixture effect for three different plant species during decomposition; (ii) to elucidate the role of β-glucosidase activity during litter decomposition; (iii) to relate the catalysis rate from the C dynamics modeling to the activity of hydrolytic enzymes.

2. Materials and methods

2.1 Study site and litter sampling

Plant litter material for laboratory and field experiments was collected at the Forbonnet peatland in Frasne (France, N46°49’35” E 6°10’20”, 840m). The site is a *Sphagnum*-dominated peatland with a mean annual temperature of 7.5°C and annual rainfall amount of 1400 mm (Laggoun-Défarge et al. 2008; Delarue et al. 2011). The vascular plant cover mainly consists of *Eriophorum vaginatum*, *Scheuchzeria palustris*, *Andromeda polifolia*, *Vaccinium oxycoccos* and *Carex limosa* (Buttler et al. 2015) with a recent increased abundance of trees of the genus *Pinus* in response to a decline in peat water content, as observed in many bogs in the study region (Frelechoux et al. 2000). Overall, the moss layer is primarily dominated by *Sphagnum fallax* and *S. magellanicum*. Litter samples of *Eriophorum vaginatum* (E) and *Pinus uncinata* (P) were composed, respectively, of senescent leaves and needles. Litter samples of *Sphagnum fallax* (S) corresponded to the decaying part just below the green photosynthesizing apical part (Bragazza et al. 2007). After having been air-dried, sub-samples of each litter type were oven dried at 50°C for 48 hours in order to calculate the dry weight.

2.2 Laboratory experiment

Sample preparation and incubation - For the laboratory experiment, samples of one gram for seven litter types were prepared. Litter types were formed by single litter of *Sphagnum fallax* (S), *Eriophorum vaginatum* (E) or *Pinus uncinata* (P), and by the following mixtures: *Sphagnum fallax* + *Eriophorum vaginatum* (SE), *Sphagnum fallax* + *Pinus uncinata* (SP), *Pinus uncinata* + *Eriophorum vaginatum* (PE) and *Sphagnum fallax* + *Pinus uncinata* + *Eriophorum vaginatum* (SPE). Litter samples received 20 mL of peatland water overnight in order to inoculate microorganisms (Hoorens et al. 2002). The excess water was removed with a tissue and the litter samples were placed in aluminum cups with small holes at the bottom to allow air circulation. The cups were placed on a 0.4 L pot containing 20 mL of saturated solution of K_2SO_4 to maintain moist conditions. Each pot with its cup was covered with perforated aluminum foil to allow air circulation. All pots were placed in an incubator (Aralab 1200) at 20°C and 95% of relative humidity. A total of 105 litter samples (pots) were prepared in order to have 3 replicates for each of the 7 litter types and 5 periodical harvests. At intervals of 0, 1, 14, 28 and 51 days after incubation, we measured CO_2 production, water extractable organic carbon (WEOC) release, litter mass loss, β-glucosidase activities and C content in 3 replicats of each litter type.

Laboratory measurements - The CO_2 production was measured with a GMP343 Vaisala probe after placing the litter sample (pots) in a 2.34 L chamber. The measurement took 15 minutes. The slope of CO_2 increased over time within the chamber (in µmol CO_2. mol air^{-1}.sec^{-1}) and was used to calculate a cumulative C release (g C g^{-1} initial C litter). The WEOC from each sample was obtained after rinsing the incubated litter three times with 50 mL of distilled water and followed the method described in Delarue et al. (2011). The extract was filtered through a 0.45 µm membrane filter. Dissolved organic carbon (DOC) in water extract was determined with a Shimadzu TOC 5000A (Total Organic Carbon Analyzer). The particles on the 0.45 µm filter and the remaining litter were dried at 50°C during two days and then weighed to obtain the remaining mass (mass loss = initial mass – remaining mass) in g OM g^{-1} initial litter mass. The C in each litter sample was then measured with an elementary analyzer (Thermo-FLASH 2000 CHNS/O Analyzer). This normalized the three C pools in g C g^{-1} initial C litter, thus making possible to input data in the C dynamics models. The activity of β-glucosidase was measured by adding to 0.5 mL of water extracted litter, 3.5 mL of 4-Methylumbelliferyl β-D-glucopyranoside, a substrate for β-glucosidase activities which is transformed into 4-Methylumbelliferone (MUF). After one hour of incubation, the concentration of MUF was determined by fluorescence using an excitation wavelength of 330 nm and an emission wavelength of 450 nm and was compared to standard solution. Measured values obtained in the litter mixtures were compared to calculate additive values (expected) after Hoorens et al. (2010), which are means of the values as measured in the decomposition of the single species litter type.

C dynamic modeling - The experimental design was similar to that published by Gogo et al. (2014, 2016). These authors conceptualized the model assuming that solid OM is catalyzed by exo-enzymes, leading to soluble OM, which is then absorbed by microorganisms and used as an energy source for different microbial functions (enzyme production, maintenance, growth). Then, the soluble organic C is respired and released into the environment in the form of CO_2. The applied model followed Schimel and Weintraub (2003) but was simplified to make it experimentally testable (Supplementary Fig. S6). It is composed of three compartments: (i) the “L” compartment corresponding to the C fraction contained in the litter (solid fraction), (ii) the “W” compartment corresponding to the C fraction in the WEOC (dissolved fraction) and (iii) the “G” compartment corresponding to the C fraction in the cumulative CO_2 released by microbial respiration (gaseous fraction). C flow from the L to the W compartment at a rate corresponding to the exo-enzyme catalysis rate. C flows from the W to the G compartment at a rate corresponding to the respiration rate. Equations describe the simultaneous change in time of the state variable (L, W, and G) and the reaction rates (Gogo et al. 2014; Supplementary Fig. S6). At any time, the sum of all these three fractions is equal to 1. The three fractions corresponding to C stocks (solid, soluble, gaseous) were experimentally measured. The catalysis rate “c” and the respiration rate “r” were tuned at the same time to fit the stock values of the model to those experimentally assessed. When the reaction rates are allowed to change in the course of the experiment, the goodness of fit is improved. The reaction rates were allowed to follow a negative exponential decrease with time. The following parameters describe the shape of the curve: “a+b” = the initial reaction rate, “a” = the final rate, “m” = decay rate with time of the reaction rate (Rovira and Rovira, 2010). Observed rates obtained in litter mixture were compared to calculate additive rates, which are means of the catalysis and respiration rates measured in decomposition of the single species litter type. The root mean square error (RMSE) was calculated for each litter type in order to represent model performance. The RMSE is calculated by squaring individual errors, summing them, dividing the sum by their total number (N), and then taking the square root of this quantity:

$$RMSE \sqrt{\sum \frac{\left(y_{pred} - y_{meas}\right)^{2}}{N}}$$

The RMSE was normalized to the mean of observed data and multiplied by 100 to obtain the Normalized Root Mean Square Error (NRMSE) in percentage (%): $NRMSE = 100 \times \frac{RMSE}{\bar{y}}$

2.3 Field experiment

The litterbag experiment was performed at the Forbonnet peatland in order to annually measure mass loss and CO_2 production during three years of field incubation. Litterbags (0.5 mm mesh) were prepared with one gram of litter of S, P, E and in a mixture of the three species (SPE). For the SPE litter mix, each species contributed equally to the final weight of the litter bag. Litter bags were placed vertically in the moss carpet and buried at 5 cm depth on November 2009 in lawns, which represent the major feature in this bog, avoiding hummocks where there was tree encroachment. Twelve samples were prepared for each of the four litter treatments that were periodically collected after one, two and three years of incubation. Litter mass loss was calculated as percentage of remaining mass of initial litter mass. The CO_2 flux was measured immediately after collection in a 835 cm^3 chamber with a Li-Cor LI-8100 analyser and expressed as fluxes (in $\mu g\ CO_2\ h^{-1}\ g^{-1}$ dry weight).

2.4 Statistics

The role of litter mixture in affecting the measured variables was compared to the corresponding additive calculated values (expected) from the litter decomposition of each single species. Two-way ANOVA was applied to investigate differences in the measured variables for both the field and laboratory experiments where the selected factors were “litter types” (observed vs additive) and “time (years or days) of measurements. Post-hoc Tukey’s Honestly Significant Differences (HSD) tests were performed to determine significant differences within groups. Differences were considered as a trend for $p < 0.1$ (noted with: $^{-}$) and significant at $p <0.05$ and referred to by * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$. Modeled curves for temporal changes of variables in the laboratory incubation experiment were calculated from the C dynamics model. All statistical analyses were done using R 3.1.1 software.

3. Results

3.1 Laboratory experiment

During the laboratory incubation of the single litter type, *Sphagnum fallax* (S) litter was characterized by a mass loss, DOC release and CO_2 production significantly different compared to *Pinus uncinata* (P) and *Eriophorum vaginatum* (E) litter (Fig. 1). The S, E and P litters contained, respectively, 42.4, 50.9 and 47.7 % of C (Table 1). These contents were constant in time and between additive (calculated values) and measured values in mixture litter (Table 1). When compared to the decomposition calculated from the single plant litter (additive mean values), the observed *Sphagnum* + *Pinus* + *Eriophorum* (SPE) mixture showed a significantly higher mass loss (i.e. lower remaining C) ($p<0.001$), a higher WEOC ($p<0.05$) as well as a higher production of CO_2 (p: 0.063) (Fig. 2). The litter mixture of *Sphagnum* + *Eriophorum* (SE) significantly ($p<0.001$) increased mass loss and CO_2 production by 13% after 51 days of incubation, as compared to the additive effect (Fig. 2, Table 2). The C dynamics in the SP and PE mixtures did not significantly differ from the additive effect of the single species (Fig. 2, Table 2) although the litter mixture of *Pinus* and *Eriophorum* (PE) showed a tendency to decrease litter mass loss and CO_2 production (p: 0.069 and 0.097, respectively).

3.2 Field experiment

After three years of field incubation, the decomposition of single litter of *S. fallax* was slower than that of vascular plants ($p<0.001$) (Fig. 3, Table 3). *S. fallax* litter showed significant differences for CO_2 production compared to *Pinus uncinata* ($p<0.001$) and *Eriophorum litter* ($p <0.05$). There was a significant effect of litter mixture during field decomposition, with a mass loss higher (ca. 19%) than that expected from the single species additive effect ($p<0.01$) (Fig. 4), while the CO_2 production in the mixture litter did not differ from the additive effect (Fig. 4, Table 3).

3.3 Catalysis of C dynamics models and β-glucosidase activities

The model fitted well the measured values with a lower NRMSE (sum between 2.82 and 9.41 %; Supplementary Table S5) compared to Gogo et al. (2014). The catalysis rate decreased with incubation time in each litter type (Table 4). In *Sphagnum* litter, this rate was initially very fast and decreased rapidly to become slower than vascular plant litters. The catalysis rates measured in *Sphagnum-Eriophorum* (SE) and *Sphagnum-Pinus-Eriophorum* (SPE) mixtures were always greater than expected from the mean of single litter species at each time of measurement (Table 4). This corresponded to higher C mass loss, WEOC and CO_2 production.

At the beginning of incubation, β-glucosidase activity was similar in all litter types but afterward it significantly increased in *Eriophorum* and *Pinus* litters, while remaining constant in *Sphagnum* litter (Supplementary Fig. S7). Mixing litters did not affect β-glucosidase activities so that there were no significant differences between observed and calculated additive values (Fig. 5). Overall, β-glucosidase activities increased with incubation time, whereas the catalysis rate decreased (Fig. 5; Table 4) so that the catalysis rate was inversely correlated to the β-glucosidase activity (r^2: 0.52; $p<0.001$; Supplementary Fig. S8).

4. Discussion

4.1 Occurrence of a synergistic effect

The laboratory incubation experiment clearly showed that the litter mixture of *Sphagnum-Eriophorum* as well as *Sphagnum-Pinus-Eriophorum* had a synergistic effect on mass loss and CO_2 production compared to the corresponding additive effect. Similar results were obtained with a mixture of *Sphagnum* litter and graminoid species (Hoorens et al. 2002; Gogo et al. 2016). Such synergistic interaction on decomposition has been explained by differences in litter chemistry such as N concentration (Hoorens et al. 2002). However, similar N concentration was found in our litter types. Mixing different litters can produce both chemical diversity and microhabitat complexity so supplying an increased diversity of substrates to the decomposers (Gartner and Cardon, 2004). Also, special attention in future studies should be devoted to the improvement of microclimatic condition such as the water content of individual litter in mixture through water flow from the wettest to the driest litter. The hypothesis is that through such water flow the conditions are improved in the driest litter, without decreasing to large extent the conditions of the wettest litter (Gogo et al., 2017).

The field experiment also showed an increase of decomposition by mixing *Sphagnum* + *Eriophorum* + *Pinus* litter. *Sphagnum fallax* decomposed at a slower rate than *Pinus uncinata* and *Eriophorum vaginatum* litter as observed elsewhere by Hobbie (1996). Curiously, we expected to have higher CO_2 production in combination with higher litter mass loss, but, instead, the highest CO_2 production was measured in *Sphagnum* litter with the slowest mass loss. The high capacity of *Sphagnum* litter to maintain capillary water could have contributed to retaining water that had previously percolated through the upper photosynthetically active centimeters of the *Sphagnum* carpet. This percolating water may have been enriched in labile C that could stimulate CO_2 production. Furthermore, the capillary structure of *Sphagnum* is known to host rich microbial communities, including microbial predators, i.e. amoebae (Jassey et al., 2013) in the living apical part and we cannot exclude the possibility that this might have affected the decaying part in the long field run. This could explain the decoupling observed between mass loss and respiration rate.

Laboratory incubation was a short experiment in which environmental factors and C input and output were well controlled. Conversely, field experiments spanned over three years and thus represent litter mass loss as it occurs in natural field conditions. In both laboratory and field experiments, mixing *Sphagnum* with *Eriophorum* and *Pinus* litter had a synergistic effect on litter decomposition. Such non-additive effects have already been reported for other ecosystems (Wu et al. 2013; Zhang et al. 2014), although the reasons are still unclear. Many studies have tried to explain such an effect by nutrient exchanges between litters (Vos et al. 2013), litter chemical quality (Meier and Bowman 2010) or changes in habitat characteristics (Lecerf et al. 2011). Nevertheless, only very few studies have addressed this effect at microbial scale and established links between microbial communities and litter decomposition (Chapman et al. 2013).

4.2 Role of β-glucosidase in early C dynamics the early decomposition stages???

Contrary to our hypothesis, no significant increases were noticed in β-glucosidase activity between the observed and the additive values during the laboratory experiment, suggesting that, at least in the early stages of decomposition (i.e. the first 28^{h} days), the synergistic mixture effect does not originate from the stimulation of this hydrolytic enzyme.

By comparing the measured enzymatic activities to the modeled catalysis rates, the *Sphagnum* litter showed values similar to those obtained by Gogo et al. (2014) and Gogo et al. (2016), with a fast rate in the early stage of decomposition, and slower but constant values thereafter (< 0.002 $gC.g^{-1}C.d^{-1}$). Contrary to our hypothesis, a negative link was established between modeled catalysis rates and β-glucosidase activities. Such a link between litter enzyme activities and decomposition rates was already assessed with hydrolases and the results showed that this relationship was weak (Allison and Vitousek, 2004). These authors suggested that mass loss occurred independently of enzyme activities with compounds that may leached out of the litter. As this last one, the really different litter types could not allowed to connect the enzymes activities with the decomposition rates. Furthermore, fast catalysis rate in the initial decomposition stage was not related to the β-glucosidase activity but maybe more to the phenol oxidase activity particularly in the case of *Sphagnum* litter (Sinsabaugh et al. 2002). Indeed, it has been shown that phenol oxidase enzymes by degrading inhibitory phenolic compounds, allow other enzymes to act on soil organic C and as a consequence regulate the stability of a vast C store in the soil following the hypothesis of ‘enzyme latch’ mechanism (Freeman et al., 2001).

Linking microbial extracellular enzyme production and litter decomposition modeled catalysis rates could provide information on the mechanisms of decomposition and non-additive effect (Sinsabaugh et al. 2002). However, using different litter types (which can change litter quality, nutrient availability and pH) such a correlation could not be demonstrated. Enzymatic degradation is still necessary to degrade complex and insoluble litter compound and relating modeled catalysis rates and enzymatic activities are likely to provide powerful models that remains poorly understood.

4.3 Sensitivity of the WEOC compartment

Mixing *Sphagnum* with *Pinus* litters did not increase OM decomposition but affected the WEOC compartment, i.e. the dissolved C pool in the laboratory-based experiment. This dissolved fraction described as the pool that is mostly sensitive to any modification in litter decomposition (e.g. Gogo et al. 2014) is considered as a transitory compartment between solid and gaseous pools, which contains a low C-content compared to the other pools. Thus, an increase in litter decomposition would have a strong effect on this compartment as observed for the *Sphagnum-Eriophorum-Pinus* litter mixture. The *Sphagnum-Eriophorum* mixture did not increase the WEOC content as this pool is a 'dynamic' compartment that is rapidly transformed into a gaseous C pool. The WEOC increase in *Sphagnum-Pinus* litter could indicate a modification in litter decomposition, which was still not perceived in the solid or gaseous compartments however.

4.4 Implication in a scenario of vegetation dynamics

Like Hoorens et al. (2002) and Gogo et al. (2016), our study showed that mixing graminoid and *Sphagnum* litters together induces a synergistic effect on decomposition. This has important implication for estimating litter decomposition rates in peatlands. These species-specific interactions appeared to be modulated by the changing climate through modifications of litter chemistry (Hoorens et al., 2002). In the context of global change and its role in causing a shift in the relative abundance of *Sphagnum* and vascular plants in peatlands (Dieleman et al., 2015), synergistic effects of mixed litters on decomposition is likely to play a critical role for the C dynamics at ecosystem level.

5 Conclusions

On the global scale, the C-sink capacity of *Sphagnum* peatlands could be affected by the litter mixture effect. This calls for further studies on non-additive effects of litter mixture, with a focus on elucidating the specific enzymatic mechanisms behind such interactions, with the ultimate goal of incorporating them in global biogeochemical models.

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Table captions

TAB. 1: C content (%), N content (%) and C:N ratio for the *Sphagnum* (S), *Pinus* (P), *Eriophorum* (E), *Sphagnum-Pinus* (SP), *Sphagnum-Eriophorum* (SE), *Pinus-Eriophorum* (PE) and *Sphagnum-Pinus-Eriophorum* (SPE) litters.

TAB 2. Levels of significance from the post-hoc tests for comparison of *Sphagnum-Pinus* (SP), *Sphagnum-Eriophorum* (SE), *Pinus-Eriophorum* (PE) and *Sphagnum-Pinus-Eriophorum* (SPE) litter mixture effect (additive vs observed) by incubation time on the remaining solid C, water extractable organic carbon (WEOC) and cumul of CO_2 - C production during the laboratory experiements. Asterisks represent significant differences (NS = not significant, ⁻ p< 0.1, *p < 0.05, **p < 0.01, ***p < 0.001).

TAB 3. Levels of significance from the post-hoc tests for comparison of *Sphagnum-Pinus-Eriophorum* (SPE) litter mixture effect (additive vs observed) by incubation time on the remaining solid C and CO_2 production during the field experiements. Asterisks represent significant differences (NS = not significant, ⁻ p< 0.1, *p < 0.05, **p < 0.01, ***p < 0.001).

TAB. 4: Catalysis rate (mg C .g^{-1}C.d^{-1}) obtained from the C-fluxes model over time for *Sphagnum* (S), *Pinus* (P), *Eriophorum* (E), *Sphagnum-Pinus* (SP), *Sphagnum-Eriophorum* (SE), *Pinus-Eriophorum* (PE) and *Sphagnum-Pinus -Eriophorum* (SPE). The model was calibrated with data from the laboratory incubation experiment. Calculate additive rates values are means from the catalysis rates measured in the decomposition of the single species litter.

Figure captions

FIG. 1: Mean (±SE, n=3) remaining solid C (a), water extractable organic carbon (WEOC) (b) and CO_2 - C production (c) in single species litter decomposition of *Sphagnum fallax* (○, S), *Pinus uncinata* (△, P) and *Eriophorum vaginatum* (◊, E) during the laboratory incubation. Lines represent the corresponding fited curves from the model for each type of litter. Asterisks represent significant differences (NS = not significant, ⁻ p< 0.1, *p < 0.05, **p < 0.01, ***p < 0.001).

FIG. 2: Additive (■) and observed (□) mean (±SE, n=3) of remaining solid C (i), WEOC (ii) and cumulative CO$_2$ - C (iii) of *Sphagnum*+*Pinus*+*Eriophorum* (a), *Sphagnum*+*Eriophorum* (b), *Pinus*+*Eriophorum* (c) and *Sphagnum*+*Pinus* (d) litter mixture decomposition during the laboratory incubation. Additive values are the weighed means from the values measured in the decomposition of the single species litter. Lines represent the corresponding fitted curves from the model. Asterisks represent significant differences (NS = not significant, ⁻ p< 0.1, *p < 0.05, **p < 0.01,***p < 0.001).

FIG. 3: Mean (±SE, n=12) remaining mass (a) and CO$_2$ production (b) of *Sphagnum fallax* (○, S), *Pinus uncinata* (△, P) and *Eriophorum vaginatum* (◇, E) litter from field litter bags experiment. Asterisks represent significant differences (NS = not significant, ⁻ p< 0.1, *p < 0.05, **p < 0.01, ***p < 0.001).

FIG. 4: Additive (■) and observed (□) mean (±SE, n=12) of remaining litter mass (a) and CO$_2$ production (b) from litter mixtures of *Sphagnum*, *Pinus* and *Eriophorum* (SPE) during field litter bags experiment. Additive values were calculated as the weighed mean of the values from litter decomposition of single plant species litter. Asterisks represent significant differences (NS = not significant, ⁻ p< 0.1, *p < 0.05, **p < 0.01, ***p < 0.001).

FIG. 5: Additive (■) and observed (□) β-glucosidase activities of *Sphagnum*+*Pinus*+*Eriophorum*, *Sphagnum*+*Eriophorum*, *Pinus*+*Eriophorum* and *Sphagnum*+*Pinus* litter mixtures (±SD, n=3). Asterisks represent significant differences (NS = not significant, ⁻ p< 0.1, *p < 0.05, **p < 0.01, ***p < 0.001). Only values from 0 to 28 days of incubation are given because a contamination occurred for the β-glucosidase activities samples at 51 days.

Tables

Table 1.

Composition	Litters						
	S	P	E	SP	SE	PE	SPE
C (%)	42.4	50.9	47.7	46.8	45.2	49.7	46.9
N (%)	1.8	1.7	1.9	1.6	1.9	1.9	1.7
C:N	23.7	29.2	25.8	30.1	23.3	26.9	26.8

Table 2.

		Main effect		Post hoc test				
		Litter type (expected vs observed	Time (days)	0	1	14	28	51
SP	Solid C		***					
	WEOC	-						
	Cumul CO_2-C		***					
SE	Solid C	***	***					***
	WEOC							
	Cumul CO_2-C	***	***					***
PE	Solid C	-	***					
	WEOC		***					
	Cumul CO_2-C	-	***					
SPE	Solid C	*	***					
	WEOC	*	***					
	Cumul CO_2-C	-	***					

Table 3

		Main effect		Post hoc test		
		Litter type (expected vs observed	Time (years)	1	2	3
SPE	Solid C	**	***			*
	CO_2 production		***			

Table 4

Litters	Time (days)

		0	1	14	28	51
S		7.87	6.61	1.91	1.66	1.65
P		2.57	2.54	2.18	1.84	1.40
E		4.02	3.51	2.22	2.20	2.20
SP	observed	3.44	3.33	2.34	1.84	1.54
	additive	3.05	5.18	1.83	1.62	1.61
SE	observed	3.57	3.51	2.86	2.34	1.77
	additive	3.41	3.34	2.57	2.00	1.44
PE	observed	3.79	3.5	1.87	1.62	1.59
	additive	3.47	3.26	2.08	1.88	1.85
SPE	observed	3.29	3.23	2.63	2.11	1.46
	additive	3.10	3.05	2.45	1.93	1.31

Figures

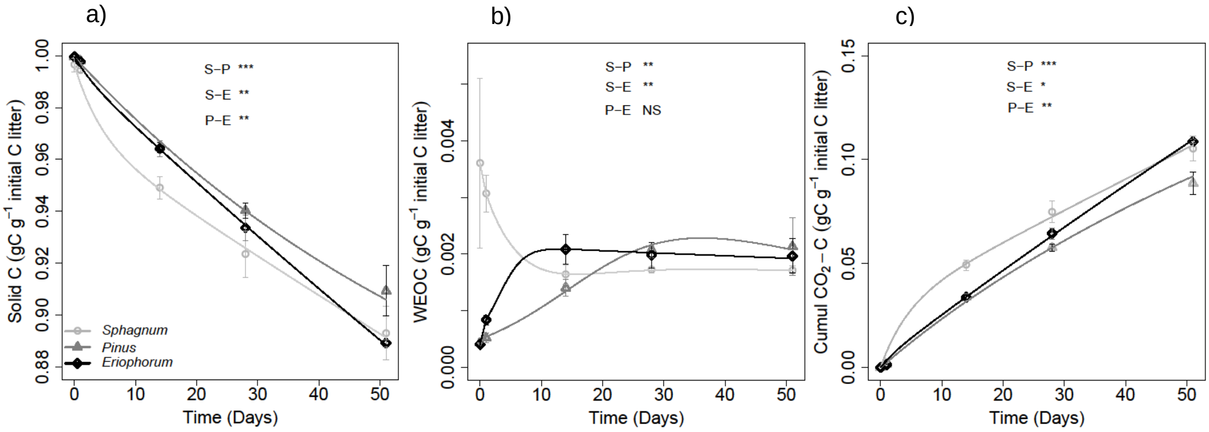


Fig. 1

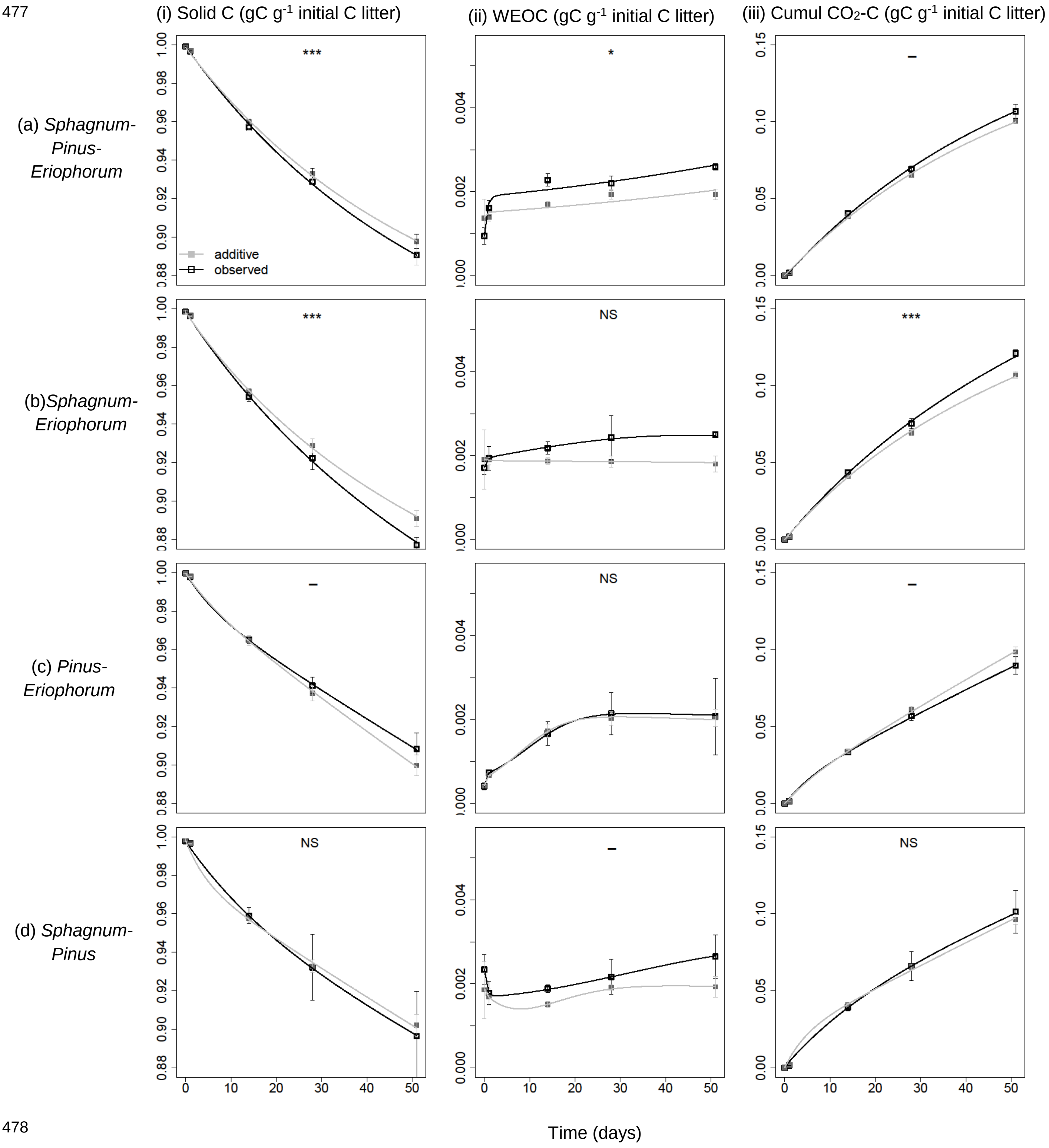

(i) Solid C (gC g-1 initial C litter)
(ii) WEOC (gC g-1 initial C litter)
(iii) Cumul CO2-C (gC g-1 initial C litter)
(a) Sphagnum-Pinus-Eriophorum
(b)Sphagnum-Eriophorum
(c) Pinus-Eriophorum
(d) Sphagnum-Pinus
additive
observed
***
*
–
***
NS
***
–
NS
–
NS
–
NS
0.88
0.90
0.92
0.94
0.96
0.98
1.00
0.000
0.002
0.004
0.00
0.05
0.10
0.15
0
10
20
30
40
50
Time (days)

Fig. 2

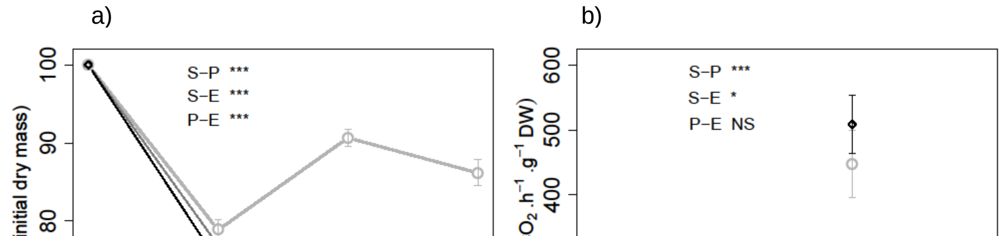

a)
b)
100
90
80
initial dry mass)
S–P ***
S–E ***
P–E ***
600
500
400
O2.h-1.g-1 DW)
S–P ***
S–E *
P–E NS

Fig. 3

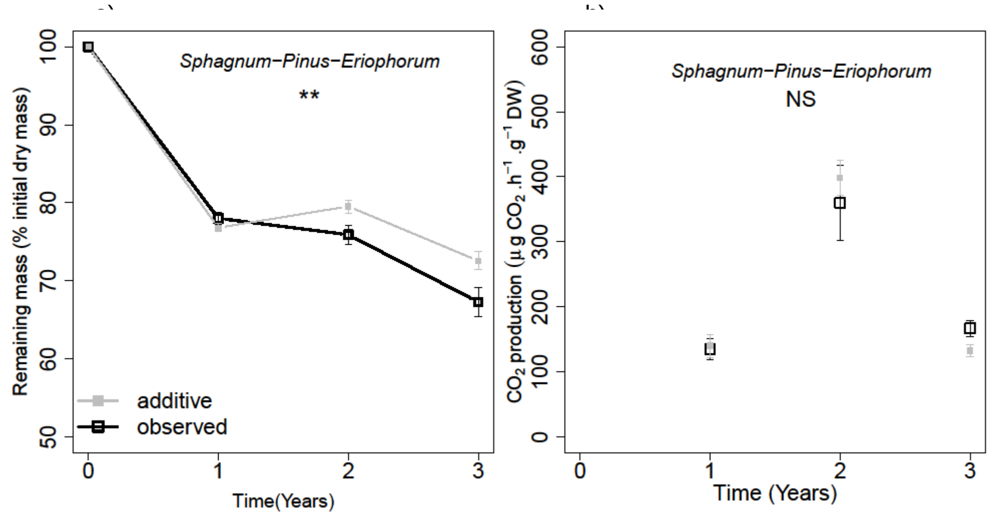

Sphagnum−Pinus−Eriophorum
**
Remaining mass (% initial dry mass)
100
90
80
70
60
50
additive
observed
0
1
2
3
Time(Years)
Sphagnum−Pinus−Eriophorum
NS
CO2 production (µg CO2 .h-1 .g-1 DW)
600
500
400
300
200
100
0
0
1
2
3
Time (Years)

Fig. 4

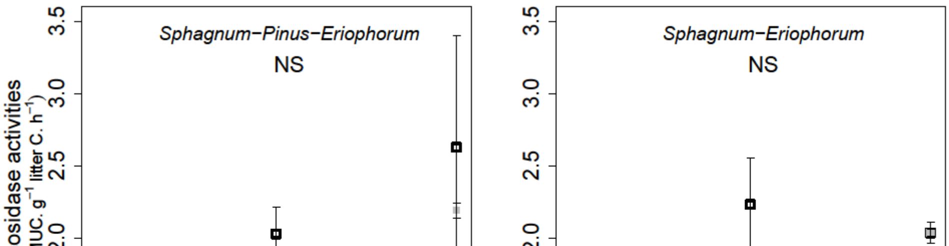

Sphagnum−Pinus−Eriophorum
NS
osidase activities
UC. g-1 litter C. h-1)
3.5
3.0
2.5
2.0
Sphagnum−Eriophorum
NS
3.5
3.0
2.5
2.0

Fig. 5

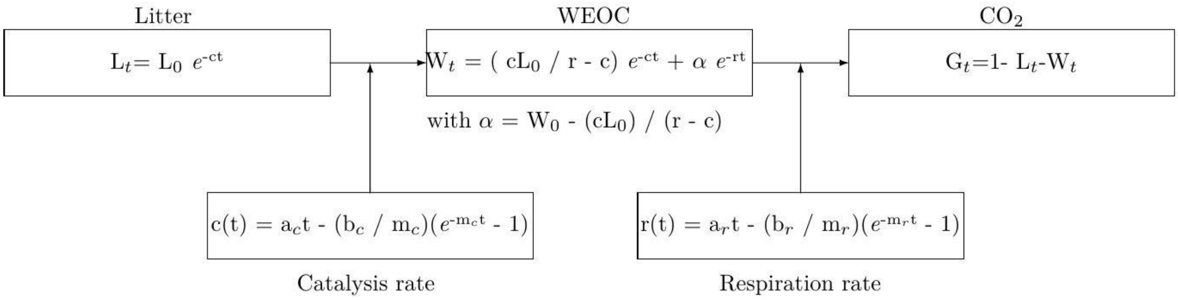
Supplementary data

Table

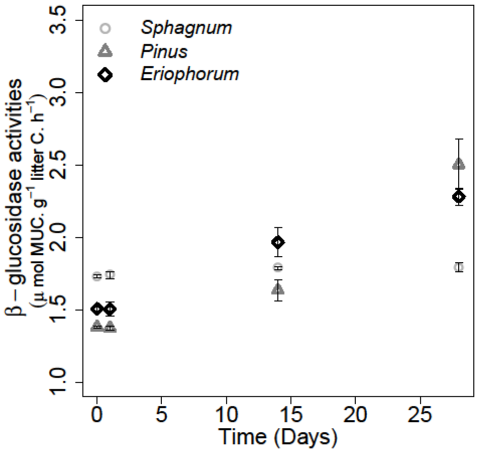
Supplementary Table S5: Percentage normalized root mean square error (% NRMSE) for the solid (L), dissolved (W), gaseous (G) pools and the sum of them for the *Sphagnum* (S), *Pinus* (P), *Eriophorum* (E), *Sphagnum-Pinus* (SP), *Sphagnum-Eriophorum* (SE), *Pinus-Eriophorum* (PE) and *Sphagnum-Pinus -Eriophorum* (SPE) models of C dynamics.

Pools	Litters						
	S	P	E	SP	SE	PE	SPE
L	0.28	0.19	0.11	0.11	0.13	0.09	0.1
W	0.28	4.08	1.89	0.19	0.48	0.84	5.54
G	5.75	5.14	2.49	2.52	2.52	2.51	1.98
Sum	6.31	9.41	4.49	2.82	3.13	3.45	7.61

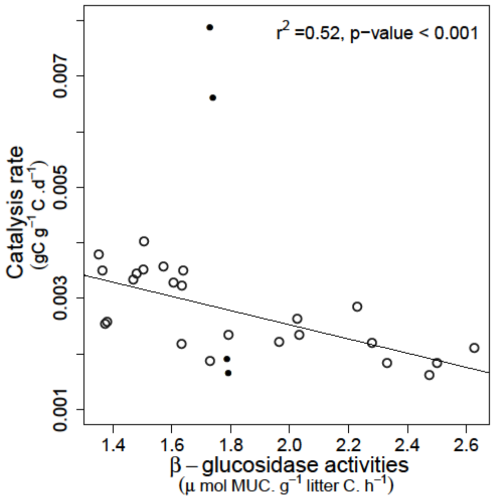
Figures



Supplementary FIG. S6: Model of the C flow in the litter decomposition process. Three compartments corresponding to the solid (litter), aqueous (WEOC), and gaseous (cumulative C-CO_2 respired) forms of C are indicated. Solid lines indicate the rates of catalysis and respiration. The L pool flows into the WEOC at the catalysis rate “c” and the WEOC is respired at the rate “r” (adapted from Gogo et al. 2014).



Supplementary FIG. S7: β-glucosidase activities in *Sphagnum* (∘), *Pinus* (△) and *Eriophorum* (⋄) litter during the laboratory incubation (±SD, n=3). Only values from 0 to 28 days of incubation are given because a contamination occurred for the β-glucosidase activities samples at 51 days.



Supplementary FIG. S8: Linear regression between catalysis rates and β-glucosidase activities. Each point represents the β-glucosidase activities measured associated to the catalysis rate obtained for each litter type at a time *t*. The r^2 was was calculated with all litters at the exception of *Sphagnum* ones (●).