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# *Juncus effusus* mono-stands in restored cutover peat bogs – Analysis of litter quality, controls of anaerobic decomposition, and the risk of secondary carbon loss



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#### ABSTRACT

Rewetting of bogs after peat extraction in eutrophied, agricultural areas often results in mono-species stands of Juncus effusus L. In contrast to typical peat forming vegetation such as Sphagnum, J. effusus is highly productive and supplies large amounts of labile carbon, but it is still unknown how this species affects biogeochemistry and the potential of restored peatlands to store carbon. To study degradation of J. effusus litter of different quality and its effect on peat decomposition, we conducted a full-factorial, anaerobic incubation with dried, <sup>13</sup>C labelled litter (roots and shoots) of fertilized (C/N ratio 19.6, C/P ratio 55.3) and non-fertilized plants (C/N ratio 57.8, C/ P ratio 304.5). We furthermore analyzed weakly (fibric) to highly (earthyfied) humified, and acidic Sphagnum-Eriophorum peat from three different rewetted and active peat extraction sites of the temperate, nemoral NW-German Plain. Materials were incubated fully water saturated at 15°C for 70 days; peat-only and litter incubations served as controls to the full-factorial re-combination of peat and litter. By this experimental design, we investigated the effect of fertilization on litter degradability, decomposition rates of litter in relation to peat, and the controls on decomposition rates to explain potential priming effects by recombination of fresh organic carbon and refractory peat. Surprisingly, fertilization of J. effusus only insignificantly affected decomposition rates between litter types. Decomposition rates of J. effusus litter were 2-18 times higher than of peat, and spectroscopic analysis of the organic matter confirmed the lability of litter and its leachates in comparison to peat. Decomposition rates were equally controlled (73% variance explained) by availability of terminal electron acceptors and the activity of hydrolyzing enzymes. However, hydrolase activity was strongly negatively correlated ( $R^2 = 0.95$ , P < 0.05) with aromaticity, suggesting an inhibitory effect. Furthermore, upon combination of litter and peat, litter was preferentially decomposed. Litter amendments in general lead to faster depletion of electron acceptors. Thus, in these anaerobic incubations, electron acceptor availability limited a priming effect on the comparably refractory peat, and importantly, input of labile carbon from J. effusus did not compromise the preservation of peat. Instead, rapid exhaustion of electron acceptors may even lead to preservation of this comparably readily decomposable plant litter.

#### 1. Introduction

Peatlands are effective sinks for atmospheric carbon (C) due to the imbalance of excess C fixation in plant biomass versus incomplete decomposition of decaying organic matter (OM) under anaerobic conditions (Vasander and Kettunen, 2006; Clymo, 1984). Peatlands store > 600 Pg C, about one third of the global soil carbon, with large impacts on atmospheric carbon and the global carbon budget (Gorham, 1991; Yu et al., 2010; Charman et al., 2013). Nevertheless, degradation of peatlands by drainage and subsequent land reclamation for agricultural, horticultural or fossil energy purposes proceeds worldwide, with about half of the wetland area already lost by anthropogenic degradation (Zedler and Kercher, 2005). Agriculture and forestry claim 80%, and peat extraction 10% of the peatland area in temperate and boreal regions (Joosten and Clarke, 2002). In Germany less than 1% of the former peatlands are still intact (Succow and Joosten, 2001; Joosten and Couwenberg, 2001). Due to an increasing awareness of the important ecosystem functions of peatlands, restoration has become an important issue. However, restoration of degraded peat bogs is challenging: the spontaneous re-establishment of peat forming vegetation (especially *Sphagnum*) in reclaimed peatlands is constrained by absence of propagules, non-optimum hydrology, non-optimum soil physical

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properties, and eutrophication (Smolders et al., 2003; Rochefort and Lode, 2006; Konvalinková and Prach, 2014). Instead more competitive vascular plants form dense mono-species stands, in central European peatlands that formerly have been used for agriculture especially soft rush J. effusus L. (McCorry and Renou, 2003; Cory and McKnight, 2005; Smolders et al., 2008). In contrast to Sphagnum, J. effusus provides a larger quantity of standing biomass exceeding 2 kg m<sup>-2</sup> dry weight (Smolders et al., 2008; Ervin and Wetzel, 1997), and production of > 7 kg m<sup>-2</sup> OM per year (Ervin and Wetzel, 2000). Despite being a perennial plant, new cohorts are produced year-round to balance die back of senescing plant parts (Ervin and Wetzel, 2000). Thus, J. effusus deposits tremendous amounts of litter that is less recalcitrant to decomposition than peat forming Sphagna (Succow and Joosten, 2001). Based on peatland area in Germany that has been used for agriculture (80%), we estimate J. effusus to cover at least 12 kha at present and this area can be expected to increase in the near future (NLWKN, 2006). Similar situations have been reported for the Netherlands and Ireland (Smolders et al., 2008; McCorry and Renou, 2003). Furthermore, J. effusus is a common plant in constructed wetlands (Means et al., 2016). Hence, the relevance of J. effusus for C cycling, sequestration, and emission of CO<sub>2</sub> and CH<sub>4</sub> needs to be addressed; J. effusus stands on peat may differ greatly from pristine bogs and might substantially alter the biogeochemistry of such sites. To assess C turnover and the potential function as carbon sink or source of these novel ecosystems it is important to evaluate processes and controls of anaerobic C-mineralization. In situ decomposition rates are on the one hand controlled by abiotic conditions such as temperature, pH, and oxygen availability. On the other hand, OM quality, nutrient availability and concentration in OM --often expressed as C/N or C/P ratios--, and microbial/enzyme activity have been identified as further controls on decomposition (Blodau, 2002). In waterlogged peatlands, organic C (OC) is largely mineralized anaerobically; this is particularly the case for restoration sites, as they are often inundated. Anaerobic OC mineralization proceeds via fermentation, and fermentation products (such as short chain fatty acids or hydrogen) are ultimately oxidized to CO2 and H2O or serve as substrates for methanogenesis (Conrad, 1996; Fenchel et al., 2013). While fermentation does not require electron transfer to an external electron acceptor (Bak and Cypionka, 1987), NO<sub>3</sub><sup>-</sup>, Mn<sup>2+</sup>/  $Mn^{4+}$ ,  $Fe^{3+}$ , humic substances, and  $SO_4^{2-}$  are sequentially utilized in terminal electron accepting processes (TEAPs) for final oxidation of OC, depending on thermodynamic energy yields (Blodau, 2011). When the total terminal electron acceptor capacity (EAC) is exhausted, methanogenesis is the predominant pathway of OC mineralization, mainly by CO<sub>2</sub> reduction with H<sub>2</sub> or cleavage of acetate (Konhauser, 2007). As methanogenesis is thermodynamically inferior to OC oxidation by TEAPs, availability of EAC is a crucial factor controlling the formation of CO<sub>2</sub> and CH<sub>4</sub>. Mineralization rates of OC are furthermore controlled by plant litter or peat OM quality, i.e. the relative amount of readily degradable components in solid (SOM) or dissolved organic matter (DOM), such as carbohydrates, free amino acids, and proteins. Together, these compounds provide a profitable energy source and are preferably decomposed (Updegraff et al., 1995; Fellman et al., 2010). By contrast, aromatic compounds, e.g. derived from complex biopolymers such as lignin and/or other secondary plant metabolites, require molecular oxygen for breakdown (Evans and Fuchs, 1988) and represent therefore recalcitrant substrates for decomposition in waterlogged peat. Hence, under anaerobic conditions, such humic substances accumulate (Freeman et al., 2001, 2004). Besides their recalcitrance, polyphenols, typically occurring among humic substances, can also act as antibiotic, fungicide, and enzyme inhibitor (Verhoeven and Toth, 1995; Freeman et al., 2004; Chomel et al., 2016). Consequently, both the intrinsic recalcitrance to degradation of humic substances and their potential inhibitory effect on enzymatic breakdown mutually impede terminal decomposition of OC. The high polyphenol content of Sphagnum is regarded as one important control for the effective preservation of its litter and of other OM as peat (Verhoeven and Toth,

1995; Verhoeven and Liefveld, 1997). The polyphenol content of J. effusus, however, might differ substantially from Sphagnum, and hence, its effect on overall decomposition. Besides litter type, it has been found that Sphagnum litter of a narrower C/N ratio, e.g. due to increased atmospheric N deposition, stimulated microbial activity and decomposition (Bragazza et al., 2006). This effect is confirmed by many studies stating that C/N ratios or C/P ratios in OM are suitable predictors for degradability (Hartmann, 1999; Bragazza et al., 2007). For plants with wide amplitudes in their nutritional requirements, such as J. effusus, it is therefore conceivable that litter decomposability also depends on the nutrient status of the specific site. Thereby, nutrient rich, labile litter may stimulate not only decomposition of fresh litter biomass but also of the fossil, refractory OM of the surrounding peat. This so-called priming effect has been demonstrated with <sup>13</sup>C or <sup>14</sup>C labeled substrate addition for mineral soils (Kuzyakov et al., 2000; Hamer and Marschner, 2002) and also in peat (Hamer and Marschner, 2002; Basiliko et al., 2012). Thus, priming is of particular interest in restored peatlands to understand processes of secondary carbon losses. However, no studies so far investigated the role of J. effusus in restored peatlands in terms of potential decomposition rates, contribution to greenhouse gas emissions, priming of residual peat, and its controlling factors. We therefore conducted a full-factorial anoxic incubation experiment to isolate the effect of nutrient availability and litter quality on litter decomposition and possible priming effects. We determined mineralization rates of three different peat types sampled in restored peatlands, of fertilized and nutrient poor <sup>13</sup>C labeled J. effusus litter, and of re-combinations of each peat and litter type. By this approach we wanted i) to identify how nutrient availability affects litter quality of J. effusus, ii) to quantify potential mineralization rates of litter derived C relative to those of peat derived C, iii) to identify major controls of mineralization, and iv) to investigate possible priming effects in a range of different OM quality from both litter and peat to assess the risk of carbon losses from restored peatlands by colonization of the non-peat forming species J. effusus. We hypothesized, that fertilization would promote the lability of the litter by increasing the amount of easily degradable carbohydrates and amino-compounds, and that mineralization rates would decrease in the order fertilized litter > non-fertilized litter > weakly decomposed peat > highly decomposed peat. Furthermore, we expected that EAC, SOM and DOM quality, nutrient concentration, solute chemistry, and activity of hydrolases would largely explain observed mineralization rates. Finally, we expected that amendments of labile OM from litter could lead to increased microbial activity and thus excess decomposition of the peat itself.

#### 2. Methods

#### 2.1. Site description and collection of peat samples

Peat samples were collected from rewetted, former cutover peatlands of "Mittleres Wietingsmoor" (N 52°64.58′55, E 8°65.05′83), "Neustädter Moor" (N 52°59.45'27, E 8°67.21'56), and in case of Vechtaer Moor (N 52°42'41.2, E 8°23'01.0) recent extraction sites within the peatland complex Diepholzer Moorniederung in the North-West German Plain. Peat formation here initiated 5600-6300 years BP (Agethen, unpublished data) on sandy soils with surface near ground water levels (Overbeck, 1975). All peatlands were extracted down to a remaining peat layer thickness of 2.1 m in the Mittleres Wietingsmoor, 1.6 m in the Neustädter Moor, and down to 0.5 m in case of the Vechtaer Moor. Peat deposits of all sites can be considered as white, fibric peat, derived from mainly Sphagnum species and a small share of Eriophorum vaginatum. However, peat quality and pore water chemistry of the material differed widely according to land use and restoration history: Peat from the Mittleres Wietingsmoor was recently formed during the last 26 years after rewetting, mainly by Sphagnum fallax and Eriophorum vaginatum, and can be considered as nutrient poor, and according to Von Post (1924) H1-H2, only weakly decomposed (WD).

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