Atmospheric nitrogen deposition promotes carbon loss from peat bogs

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Peat bogs have historically represented exceptional carbon (C) sinks because of their extremely low decomposition rates and consequent accumulation of peat remnants as peat. Among the factors favoring that peat accumulation, a major role is played by the chemical quality of plant litter itself, which is poor in nutrients and characterized by polyphenols with a strong inhibitory effect on microbial breakdown. Because bogs receive their nutrient supply solely from atmospheric deposition, the global increase of atmospheric nitrogen (N) inputs as a consequence of human cultural activities could potentially alter the chemical quality of biologically reactive nitrogen (N) from industrial and agricultural activities (6) could potentially alter the chemical quality of plant litter with consequent effects on the amount of C released during litter decomposition. Accordingly, an understanding of the mechanisms of bog soil C response to changing N availability is essential for assessing the capacity of peat bogs to continue to contribute to the net land C sink (2, 7).

To investigate the specific role of N availability in litter peat decomposition, we incubated Sphagnum litter samples in the laboratory under uniform temperature conditions, after the addition to each litter peat sample of an identical microbial inoculum. These controlled incubation conditions ensured that any difference in decomposition was purely the result of initial peat quality. To avoid confusion with short-term N fertilization effects, we collected litter peat samples under a natural gradient of chronic N depositions, selecting 12 bogs from nine European countries spanning a range from 0.2 to 2 g of Nm−2 yr−1. Differences in litter peat chemistry along the gradient of atmospheric N deposition were assessed, determining total N, C, phosphorus (P), and polyphenol concentrations. The amount of C released during litter peat decomposition was assessed by measuring carbon dioxide (CO2) emission and dissolved organic carbon (DOC) concentration in association with the activity of the main hydrolase and oxidase enzymes.

Results and Discussion

Atmospheric N deposition at each bog correlated positively with the mean N/P ratio (r = 0.79, P < 0.01; n = 12) but negatively with the mean C/N ratio (r = −0.71, P < 0.01; n = 12) in the litter, indicating that chronically increased N inputs affect litter peat chemistry through a higher accumulation of N compared with P and C. Higher N concentrations in litter peat stimulated short-term decomposition as supported by increasing trends of CO2 emission (Fig. 1). We propose a direct and an indirect effect of increased N availability on litter peat decomposition to be invoked. The direct effect is due to alleviated N limitations on microbial metabolism, which is then stimulated by decreasing C/N ratios of litter peat (8–10). The above mechanism is supported by our observation of decreased activity of chitinase, an enzyme associated with degradation and release of N from organic matter, along the gradient of N deposition (Fig. 2).

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Abbreviations: DOC, dissolved organic carbon; MUF, methylumbelliferyl.

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polyphenols by living Sphagnum from litter with a lower C/N ratio) reflects a lower production of samples collected under higher atmospheric N deposition (i.e., ).

Shipas explained by a logarithmic regression for both incubation periods .

Because polyphenols play a pivotal role in inhibiting microbial enzymatic activity (15), a protein and polyphenol synthesis (14). Because polyphenols play a pivotal role in inhibiting microbial enzymatic activity (15), a lower initial occurrence of polyphenols in litter peat indirectly promotes microbial decomposition (16, 17).

The indirect effect of N availability is related to the negative relationship between initial polyphenol concentration in litter peat and atmospheric N deposition ( ), . A lower release of polyphenols from litter peat samples collected under higher atmospheric N deposition (i.e., from litter with a lower C/N ratio) reflects a lower production of polyphenols by living Sphagnum plants when N availability is high, in accordance with an inverse relationship between rates of protein and polyphenol synthesis (14). Because polyphenols play a pivotal role in controlling litter peat decay. Indeed, because the activity of an extracellular enzyme is regulated by the availability of the nutrient that the enzyme mineralizes (11–13), the decreasing activity of chitinase with increasing N concentration in litter peat supports our view that we are observing a reduction of N limitation on microbial metabolism.

The indirect effect of N availability is related to the negative relationship between initial polyphenol concentration in litter peat and atmospheric N deposition ( ). A lower release of polyphenols from litter peat samples collected under higher atmospheric N deposition (i.e., from litter with a lower C/N ratio) reflects a lower production of polyphenols by living Sphagnum plants when N availability is high, in accordance with an inverse relationship between rates of protein and polyphenol synthesis (14). Because polyphenols play a pivotal role in inhibiting microbial enzymatic activity (15), a lower initial occurrence of polyphenols in litter peat indirectly promotes microbial decomposition (16, 17).

The direct and indirect effects of N availability on litter peat decomposition are connected through the activity of phenol oxidase, one of the few enzymes able to degrade polyphenols. The contrasting effects of N additions across different studies on phenol oxidase activity are primarily explained by the amount of lignin in plant litter (18–21), with species low in lignin showing a high activity and species high in lignin showing a low activity of phenol oxidase. Our finding of an increased activity of phenol oxidase with increasing N deposition (Fig. 3) is in accordance with the absence of lignin in Sphagnum plants (3), although these mosses contain peculiar, decay-resistant polyphenols (5, 22). An enhancement of phenol oxidase activity reduces polyphenol concentrations during litter peat decay (Fig. 3) and, indirectly, stimulates the activity of hydrolase enzymes (Fig. 2) via the phenol oxidase latch mechanism (15, 16) with an additional ultimate positive feedback on decomposition rate.

When nitrogen deposition is higher than 1 g m$^{-2}$ yr$^{-1}$, CO$_2$ emission shows a saturating trend (Fig. 1) that can be explained by an increasing P limitation on microbes (23, 24), as supported by the very steep increase in phosphatase activity (Fig. 2). In this case, the negative relationship between phosphatase and chitinase reflects a tradeoff in microbial resource allocation between two different enzymes when nutritional constraints change (12, 25), suggesting that, with high N deposition, P availability plays a primary role in controlling litter peat decay.

We also found a further potential C loss through an increased release of DOC from litter peat collected under higher N deposition (Fig. 4). We suggest that the increase of DOC concentration was a consequence of enhanced enzymatic activity, in particular of $\beta$-glucosidase (24, 26, 27), an extracellular enzyme involved in the release of C from organic matter. This biologically driven mechanism is supported by the significantly higher DOC concentration values at the end of the incubation compared with the correspondent initial amount of DOC released (Fig. 4), in accordance with increasing activity of $\beta$-glucosidase (Fig. 2). In addition, the absence of a relationship between DOC concentration and N deposition before the start
of the incubation \((r = 0.44, P = 0.16; n = 12)\) and the correspondent positive correlation after 10 days of incubation \((r = 0.76, P < 0.01; n = 12)\) support the primary role of enhanced metabolic activity of microbes in affecting the release of DOC. Furthermore, the role of enzymatic activity, i.e., a primary biological control over DOC production, is consistent with the negative correlation between molecular weight of dissolved organic matter and \(\beta\)-glucosidase activity \((r = -0.64, P = 0.03; n = 12)\) as well as with the positive correlation between \(\mathrm{CO}_2\) emission and final DOC concentration \((r = 0.63, P = 0.03; n = 12)\) \((28, 29)\).

Increasing \(N\) availability can promote plant productivity in bogs in locations where \(N\) remains a limiting nutrient \((30–32)\). However, \(Sphagnum\) productivity has only been shown to respond positively to atmospheric \(N\) deposition until a critical threshold of \(~1\, \text{g} \cdot \text{m}^{-2} \cdot \text{yr}^{-1}\), beyond which productivity is reported to decrease \((30, 33–35)\). Moreover, increased \(N\) deposition may also give a competitive advantage to vascular plants \((30, 36)\) whose litter is more easily decomposed \((37)\). However, as \(Sphagnum\) remains the dominant peat-building component of European bogs, our findings indicate that high chronic \(N\) additions have the potential to promote peatland \(C\) release in gaseous and aqueous forms. The extent to which any future expansion of vascular plants might offset the reduced \(Sphagnum\) plant productivity and increased litter peat decomposition remains an important topic for future research. Such information would be a prerequisite for more precise prediction of the future peatland \(C\) storage capacity. Nevertheless, our results clearly highlight an urgent need for policy responses to combat global \(N\) eutrophication if we are to avoid the risk of a potential destabilization of these globally significant \(C\) stores.

**Materials and Methods**

**Study Bogs.** We selected 12 European bogs spanning a latitudinal gradient from \(\sim 45^\circ \text{N} \) to \( \text{63}^\circ \text{N} \) and a longitudinal gradient from \(\sim 5^\circ \text{W} \) to \(17^\circ \text{E}\). In particular, one bog was selected in Norway \((n = 3\text{ peat samples})\), Finland \((n = 3)\), Great Britain \((n = 3)\), The Netherlands \((n = 6)\), Switzerland \((n = 6)\), and Slovenia \((n = 6)\), whereas two bogs were selected in Sweden \((n = 3 \text{ per bog})\), Czech Republic \((n = 6 \text{ per bog})\) and Italy \((n = 6 \text{ per bog})\). Data on atmospheric \(N\) deposition at each bog were obtained from national organizations responsible for precipitation monitoring \((38)\) and refer to the 3 years preceding litter peat sampling. The natural gradient of chronic atmospheric \(N\) deposition spanned a range from \(0.14 \text{ g} \cdot \text{m}^{-2} \cdot \text{yr}^{-1}\) \((\text{Sweden})\) to \(2 \text{ g} \cdot \text{m}^{-2} \cdot \text{yr}^{-1}\) \((\text{The Netherlands})\). For a detailed description of study bogs and sampled habitats see Bragazza *et al.* \((38)\).

**Field Sampling.** At each study bog, litter peat samples were collected in similar plant communities and microtopographic positions (hummocks and lawns) as previously described \((35)\). The sampling was performed in areas with a dense cover of healthy \(Sphagnum\) plants and a very sparse, if any, cover of vascular plants. Hummocks had \(Sphagnum\) fuscum \((\text{Schimp.})\) \(\text{Klinggr.}\) and/or \(Sphagnum capillifolium\) \((\text{Ehrh.})\) Hedw. as dominant species, whereas lawns were mostly dominated by \(Sphagnum\) magellanicum \(\text{Br.}\) A total of 30 hummocks and 27 lawns were available \((\text{see above})\). Because decomposition of \(Sphagnum\) litter is a continuous process, we chose recently formed \(Sphagnum\) litter \((\text{hereafter called } \text{“litter peat”})\) at \(\sim 2–4\text{ cm below the bog surface} \) in accordance with previous studies \((39, 40)\).

**Litter Peat Incubation and \(\text{CO}_2\) Measurements.** Remnants of vascular plants were removed from litter peat samples before incubation. A microbial inoculum was prepared by mixing 0.5 kg of fresh \(Sphagnum\)-dominated litter peat with 1.5 liters of deionized water. After 30 min of stirring, the slurry was filtered through a coarse glass filter and used to inoculate the collected litter peat samples. Approximately 0.1 g of air-dried litter peat was aerobi- cally incubated with 8 ml of slurry in glass vials in darkness at a constant temperature of \(12^\circ \text{C}\) and shaken frequently. After 4 and 10 days, the glass vials were sealed, and evolved gases were allowed to accumulate for 1 h. A gas sample was then collected from each glass vial by using a gas-tight syringe, and evolved \(\text{CO}_2\) concentrations were analyzed on a gas chromatograph \((\text{AMS Model 92, Analytical Measuring Systems, Cambridge, U.K.})\). Concentrations were then converted to \(\text{CO}_2\) production per initial gram of litter peat \((\text{dry weight}; \text{see below})\) and per hour of incubation, taking into account the \(\text{CO}_2\) concentration produced by the slurry used for the inoculum \((\text{six replicates})\).

**Chemical Analyses.** Total concentration of \(N\) and \(P\) in litter peat samples was determined colorimetrically on a flow-injection autoanalyzer \((\text{FlowSys, Systea, Anagni, Italy})\) after acidic digestion by following the Kjeldahl procedure. Litter peat \(C\) concentrations were determined on an elemental analyzer \((\text{EA 1110, Carlo Erba, Milan, Italy})\). Litter peat subsamples were dried at \(40^\circ \text{C}\) for 24 h to convert all concentrations to oven dry weight. \(\text{DOC}\) concentrations were determined as the difference between total \(C\) and inorganic \(C\) by using a total \(C\) analyzer \((\text{Shimadzu 5000, Kyoto, Japan})\). Initial \(\text{DOC}\) concentration released from each litter peat sample was determined after stirring \(\sim 0.1\) g of air-dried peat in 8 ml of distilled water for 4 h. After 10 days of incubation, final \(\text{DOC}\) concentrations were determined in the extract formed by the slurry plus the litter peat. Before \(\text{DOC}\) and polyphenol analyses, all samples were filtered through a 0.45-\(\text{m} \) glass filter. \(\text{DOC}\) concentration present in distilled water and in the slurry was taken into account in the calculation. The initial and final concentrations of polyphenols were determined in the same solution used for \(\text{DOC}\) by following the Folin–Ciocalteu procedure \((26)\). Polyphenol concentrations in distilled water and in the slurry were taken into account in the calculation. Both \(\text{DOC}\) and polyphenols were expressed per initial gram of litter peat \((\text{dry weight})\).

**Enzymatic Assays.** After 10 days of incubation, each litter peat sample was assayed for the activity of hydrolases \(\beta\)-glucosidase, \(\text{chitinase} [\text{N-acetylglucosaminidase (NAGase)}], \) and...
phosphatase, as well as for the activity of oxidative phenol oxidase (15). The activity of hydrolase enzymes was detected for each litter peat sample by adding 4-methylumbelliferyl (MUF)-β-D-glucoside for the activity of β-glucosidase, 4-MUF-N-acetyl-β-D-glucosaminide for the activity of chitinase, and 200 μM MUF-phosphate for the activity of phosphatase to an aliquot of extract (peat plus slurry). After incubation (1 h for β-glucosidase and chitinase and 45 min for phosphatase) at 12°C, the fluorescence of the supernatant was measured immediately on a microplate reader (FLUOstar Galaxy, BMG Labtechnologies, Offenburg, Germany) at 450-nm emission and 330-nm excitation wavelength. To quantify product release and account for quenching effects, a set of standards was prepared according to the above procedure (methylumbelliferone replaced the MUF substrates). Enzyme activities were expressed as μmol of substrate (MUF) converted per minute and per gram (dry weight) of litter peat. The activity of phenol oxidase was determined spectrophotometrically by using 10 mM L-dopa (dihydroxyphenylalanine) solution as substrate. The activity of phenol oxidase was expressed as μmol of 2,3-dihydroindole-5,6-quinone-2-carboxylate (dicq) h⁻¹ g⁻¹ litter peat (dry weight).

**Spectroscopic Measurements.** The quotient of wavelength absorption at 365 nm and 250 nm was used as the indication of molecular weight of dissolved organic matter (41).

**Statistical Analyses.** A multiple backward regression of CO₂ emission (after 4 days) against N deposition, latitude, elevation of the bog, mean annual temperature, and annual precipitation was initially performed. N deposition was identified as the only significant predictor variable (\( F_{1,10} = 14.9, P = 0.003, R^2_{\text{adjusted}} = 0.56 \); standardized regression coefficient for N deposition = 0.26, \( P < 0.01 \) accounting for differences in litter decomposition compared with climatic conditions and geographical position. All statistical analyses were performed after averaging the hummock and lawn data of the same bog. Pearson correlations (r) and univariate regressions (R²) were used.

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1. Moore TD (2002) Environ Conserv 29:3–20.
2. Smith LC, MacDonald GM, Velichko AA, Beilman DW, Borisova OK, Frey KE, Kremenetski KV, Sheng Y (2004) Science 303:353–356.
3. van Breemen N (1995) Trends Ecol Evol 10:270–275.
4. Painter TJ (1991) Carbohydr Polym 15:123–142.
5. Rudolph H, Samland J (1985) Phytochemistry 24:745–749.
6. Galloway JN, Vitousek PM (2000) Biochemistry 12:153–226.
7. Scholes RJ, Noble IR (2001) Science 294:1012–1013.
8. Limpens J, Berendse F (2003) Oikos 103:537–547.
9. Hu S, Chapin FS, III, Firestone MK, Howarth RW, Seitzinger SP, Asner GP, Cleveland CC, Green PA, Holland EA, et al. (2004) Biogeochemistry 70:153–226.
10. Knorr M, Frey SD, Curtis PS (2005) Ecol Appl 15:1172–1177.
11. Allison SD, Vitousek PM (2005) Biogeochemistry 70:153–226.
12. Olander LP, Vitousek PM (2000) Biogeochemistry 49:175–190.
13. Rudolph H, Samland J (1985) Phytochemistry 24:745–749.
14. Freeman C, Ostle NJ, Capone DG, Boyer EW, Howarth RW, Seitzinger SP, Asner GP, Cleveland CC, Green PA, Holland EA, et al. (2004) Biogeochemistry 70:153–226.
15. Scholes RJ, Noble IR (2001) Science 294:1012–1013.
16. Limpens J, Berendse F (2003) Oikos 103:537–547.
17. Hu S, Chapin FS, III, Firestone MK, Howarth RW, Seitzinger SP, Asner GP, Cleveland CC, Green PA, Holland EA, et al. (2004) Biogeochemistry 70:153–226.
18. Andersson S, Nilsson SJ, Sætren P (2000) Soil Biol Biochem 32:1–10.
19. Berendse F, van Breemen N, Rydin H, Buttlar A, Heijmans M, Hoosbeek MR, Lee JA, Mitchell E, Saarinen T, Vasander H, et al. (2001) Global Change Biol 7:591–598.
20. Berendse F, van Breemen N, Rydin H, Buttlar A, Heijmans M, Hoosbeek MR, Lee JA, Mitchell E, Saarinen T, Vasander H, et al. (2001) Global Change Biol 7:591–598.
21. Berendse F, van Breemen N, Rydin H, Buttlar A, Heijmans M, Hoosbeek MR, Lee JA, Mitchell E, Saarinen T, Vasander H, et al. (2001) Global Change Biol 7:591–598.
22. Verhoeven JT, Liefeld WB (1997) Acta Bot Neerl 46:117–130.
23. Turner BL, Baxter R, Whitton BA (2002) Environ Pollut 120:313–317.
24. Henry H, Juarez J, Field CB, Vitousek PM (2005) Global Change Biol 11:1808–1815.
25. Sinsabaugh RL, Carreiro MM, Repert DA (2002) Biogeochemistry 60:1–24.
26. Feener N, Freeman C, Reynolds B (2005) Soil Biol Biochem 37:1814–1821.
27. Gallo ME, Lauber CL, Cabaniss SE, Waldrop MP, Sinsabaugh RL, Zak DR (2005) Global Change Biol 11:1514–1521.
28. Jandal R, Sollins P (1997) Biol Fertil Soils 25:196–201.
29. Andersson S, Nilsson SJ, Sætren P (2000) Soil Biol Biochem 32:1–10.
30. Berendse F, van Breemen N, Rydin H, Butler A, Heijmans M, Hoosbeek MR, Lee JA, Mitchell E, Saarinen T, Vasander H, et al. (2001) Global Change Biol 7:591–598.
31. Berendse F, van Breemen N, Rydin H, Buttlar A, Heijmans M, Hoosbeek MR, Lee JA, Mitchell E, Saarinen T, Vasander H, et al. (2001) Global Change Biol 7:591–598.
32. Moore T, Blodau C, Turunen J, Routen N, Richard PJH (2004) Global Biogeochem Cycles, 10.1029/2003GB002154.
33. Moore T, Blodau C, Turunen J, Routen N, Richard PJH (2004) Global Change Biol 11:356–367.
34. Vitt D, Wieder RK, Halsey LA, Turatsyn S (2003) Biotropica 35:180–185.
35. Gunnarsson U, Rydin H (2000) New Phytol 147:527–537.
36. Limpens J, Berendse F, van Breemen N, Rydin H, Buttlar A, Heijmans M, Hoosbeek MR, Lee JA, Mitchell E, Saarinen T, Vasander H, et al. (2001) Global Change Biol 7:591–598.
37. Bragazza L, Tahvanainen T, Kutnar L, Rydin H, Limpens J, Hajek M, Grosvener M, Hajek T, Hajek P, Hansen I, et al. (2004) New Phytol 163:609–616.
38. Limpens J, Berendse F, Kleeis H (2003) New Phytol 157:339–347.
39. Bragazza L, Tahvanainen T, Kutnar L, Rydin H, Limpens J, Hajek M, Grosvener M, Hajek T, Hajek P, Hansen I, et al. (2004) New Phytol 163:609–616.
40. Bragazza L, Limpens J, Gerold R, Grosvener M, Hajek M, Hajek T, Hajek P, Hansen I, et al. (2004) New Phytol 163:609–616.
41. Bragazza L, Limpens J, Gerold R, Grosvener M, Hajek M, Hajek T, Hajek P, Hansen I, et al. (2004) New Phytol 163:609–616.