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TELLUS

Nitrogen cycling drives a strong within-soil CO_2-sink

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ABSTRACT

For about three decades, it has not been possible to completely balance global carbon emissions into known pools. A residual (or 'missing') sink remains. Here evidence is presented that part of soil respiration is allocated into an internal soil CO_2-sink localized to the saprophytic subsystem (roots excluded). The process occurs in forest, agricultural and grassland soils and is favoured by high N-deposition. Chemoautotrophic nitrification has a key role, and the most efficient internal CO_2-sequestration occurs concurrently with lowest soil nitrate ($NO_3{}^-$) concentrations, despite considerable N-loading. Not until drastic N-supply occurs, does the CO_2-sink successively breakdown, and nitrate concentrations increase, leading to $NO_3{}^-$-leaching. Within-soil CO_2-uptake seems to be of the same magnitude as the missing carbon sink. It may be gradually enforced by the ongoing input of nitrogen to the biosphere.

1. Introduction

The CO_2 increase in the atmosphere is more significantly delayed by a still unexplained terrestrial sink than any emission reduction implemented so far. The nature of this 'missing' sink has long been a mystery. Carbon accumulation in forest trees as a result of N-deposition has been suggested, but also rejected (Nadelhoffer et al., 1999) as a major explanation of the C-sink. From a geographically extensive study in areas with varying atmospheric N-deposition, lowest soil CO_2 concentrations (and therefore lowest soil CO_2 emissions) were found in high N-deposition areas, despite their higher production. These findings were the basis for the hypothesis that nitrogen cycling causes a within-soil CO_2-sink, allowing only part of CO_2 from soil respiration to leave the soil surface (Fleischer, 2003). This contradicts the previously accepted view that net ecosystem exchange of CO_2 is, by definition, solely the difference between gross photosynthesis and respiration (Baldocchi and Gu, 2003). The fate of 2–3 $\mathrm{Pg\,C\,yr^{-1}}$ in the global carbon cycle (Houghton, 2003) still needs to be explained to balance the global C-budget.

2. Materials and methods

Nitrogen-depositions were derived from the EMEP program (grid size 50 × 50 km) and from the German program on critical loads (1×1 km; Gauger et al., 2002). Exposed forest edges receive 40% higher N-deposition than the closed forest 50 m from the edge (Spangenberg and Kölling, 2004). The sites studied experimentally with $NH_4{}^+$-additions were chosen in connection with large running research projects where background data are available (Table 1). Twenty-five to forty composite soil samples were taken at each site from the 0 to 5 cm horizon (litter excluded). They were transported within ±2 °C deviation from sampling temperatures and further worked up immediately after arrival. The soil was sieved (2 mm) to eliminate coarse material and remains of roots so that the saprophytic subsystem could be studied. Successively increasing amounts of $NH_4{}^+$ were added to series of the soil samples in 12 ml exetainers (0.5–1.5 g soil, three replicates) (Fig. 1). The exetainers were left open for stabilization/acclimatization for three weeks in darkness with maintained water content (the exetainers were weighed every third day to check and adjust for moisture losses). After this acclimatization period they were closed, and the increases of the CO_2-concentrations were measured after about 1 d at 15 °C in darkness. In addition to inhibition with high $NH_4{}^+/NH_3$ (Vazquez-Rodriguez, 1997), the importance of chemoautotrophic nitrification for the soil CO_2-sink was also indicated from incubations with and without acetylene, which is specifically inhibiting this process. Acetylene-saturated water was added, the final concentration (0.8–2%) was dependent on organic material and initial water content to avoid surplus water. The internal soil CO_2 sink (%) is $[(GHR - NHR)/GHR] \times 100$, where GHR is gross heterotrophic respiration, revealed after incubation with acetylene. NHR is net heterotrophic respiration in untreated soil (root respiration excluded in this sieved soil).

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Table 1. Characteristics of the European sites, selected adjacent to ongoing research projects. They are referred to in the right-hand column

Site	Region, Country	Characteristics, distance from forest edge (m)	Long., lat.	Altitude (m a.s.l.)	N deposition 2/50 m ($\mathrm{kg\,N\,ha^{-1}\,yr^{-1}}$)	Experimental N supply $\mathrm{kg\,ha^{-1}}$ (since)	LOI (% of DW)[a]	Nearby research site descriptions, references
Hjuleberg	SW Sweden	Intensive forestry, Norway spruce, former arable land	56°58′N, 12°43′E	40	20	275 (1997)	14	Grip (2006)
Grillenburg	Saxony, Germany	Norway spruce 2/50 m	50°57′N, 13°30′E	381	84/60	No	51.4/75.8	Grünwald and Bernhofer (2007)
Sparneck	Bavaria, Germany	Norway spruce 2/50 m	50°09′N, 11°51′E	655	88/63	No	68.0/61.1	Gerstberger (2001)
Wetzstein	Thuringia, Germany	Norway spruce closed forest	50°27′N, 11°27′E	789	51	No	67.3–85.5	Anthoni et al. (2004)
Hainich	Thuringia, Germany	Old closed deciduous forest, beech, ash and others	51°04′N, 10°27′E	490	48.5	No	16.0	Mund (2004)

[a] Organic material loss on ignition (LOI).

GS-chromatography was carried out with a Varian 3300 instrument on Haye Sep Q. Reference and carrier gases were from Air Liquide Gas Company. NO_3^- in soil was determined by flow injection analysis on a FIA Star 5010 instrument, after extraction in 2 M KCl. NO_3^--concentrations at the Hjuleberg research forest were provided (Fig. 2; Grip, 2006). Plot area of the parcels was 50 × 50 m and five suction lysimeters were used at each location.

Levene tests of homogenity of variances were used to test if variances between groups were equal. If the variances were found equal the one-way ANOVA and post hoc Sidak tests were used to test if there are differences in the CO_2-concentrations for the nitrogen additions following one after the other. If variances were found non-equal the non-parametric Kruskall–Wallis tests and post hoc *U*-tests were used. The statistical analyses were performed with SPSS for Windows, Version 15.

3. Results and discussion

CO_2-uptake by chemoautotrophic nitrification in high N-deposition European soils, receiving no experimental N-additions, was inhibited with acetylene. In this way GHR was disclosed, and this made it possible to estimate the within-soil CO_2-sink from the difference of CO_2 emitted with (GHR) and without (NHR) acetylene. From September 2004 to July 2007, an average sink amounting to −11% of the heterotrophic respiration was indicated (from a strong sink of −30% in a spruce forest to a source of +8% CO_2, $n = 29$, 26 sink and 3 source values). In remote areas with less N-deposition (Sweden between lat. 56°N and 66°N, from December 2004 to September 2007), an average sink of −5% was indicated at 11 forest, 2 agricultural and 2 grassland sites (from an extreme sink of −57% at an agricultural site to a source of +15% CO_2, n=53, 35 sink and 18 source values). We assumed this to be an expression of the cyclic nature of gross nitrification with net uptake of CO_2, (see discussion on mechanism), and this deserved a further study with complementary methodology.

When 13 series of central European soils (Table 1) were further analysed a general pattern emerged (Fig. 1). Increasing amounts of NH_4^+ added to soils to increase nitrification, decreased CO_2-emissions, until a minimum was reached, representing the maximum soil CO_2-sink at the site. Further NH_4^+-supply gradually inhibited nitrification (Vazquez-Rodriguez, 1997) now resulting in increased CO_2-release and GHR was disclosed. The interpretation that the CO_2-decrease leading to the minimum is a result of inhibited or retarded respiration caused by nitrogen compounds is not feasible. Otherwise, an additional supply of NH_4^+ would have further stabilized the minimum CO_2-concentration and not resulted in significantly higher CO_2-release. An average CO_2-sink of almost 16% was indicated in this high atmospheric N-deposition area (Fig. 2).

High N-deposition areas also cover eastern North America, India, China and, partly, South America and Africa, approaching 1/4 of all land areas (Rodhe et al., 1995; Galloway et al., 2004). Providing that they exhibit a CO_2 sink in the same magnitude as indicated in central Europe (11–16%) and remote areas (5%), and a NHR of 40.2 Pg from soils (Raich et al., 2002), implying that 50% root respiration was excluded in the sieved soils (Wang et al., 2005), a tentative within-soil CO_2-sink of 2.8–3.3 Pg is indicated. This scaling up indicated the same magnitude as the

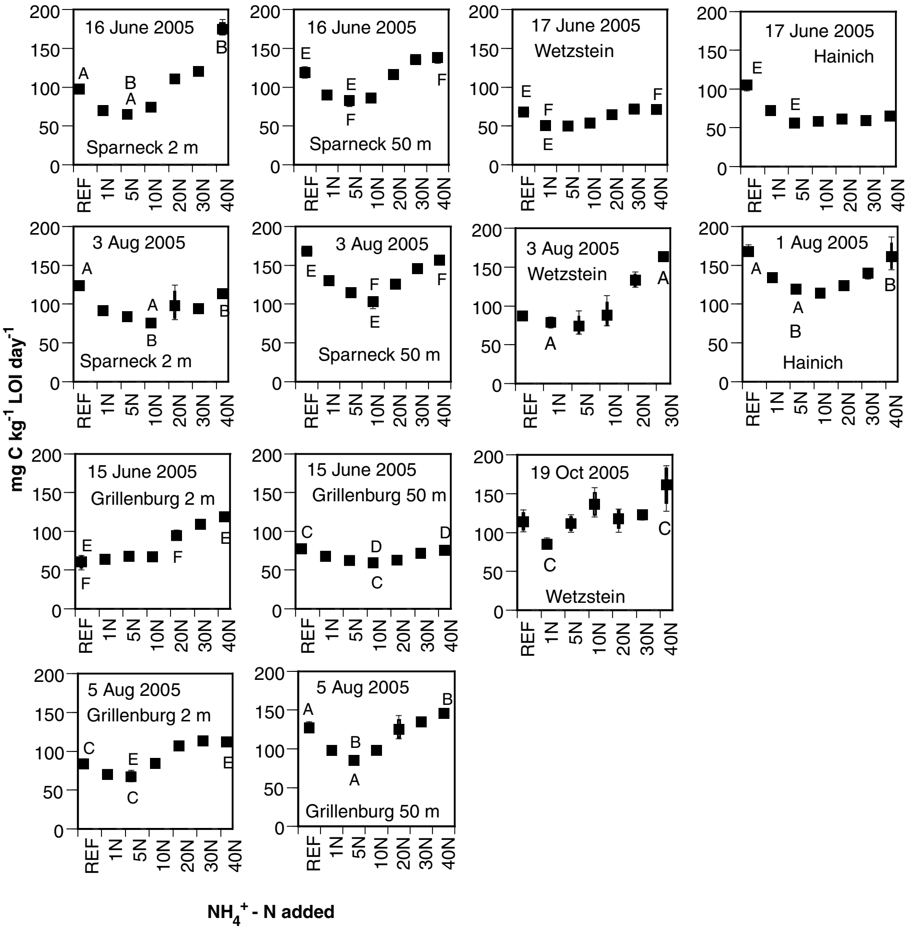

Fig. 1. Emission of CO_2 from the 0–5 cm horizon in Central European forest soils receiving high atmospheric N-deposition. REF is the original soil receiving solely atmospheric N-deposition at the site. This soil was also supplied with successively increasing amounts of NH_4^+. 1N represents addition of 2.46 mg NH_4^+–N per g of organic material (LOI, loss on ignition). $n = 3$ for each incubation, outliers shown if not hidden by the squares representing means. Values indicated A/A, B/B are significantly separated from each other, $p < 0.05$; C/C, D/D, $p < 0.01$; E/E, F/F with $p < 0.001$.

residual sink but has a potential variable affecting outcome, because the sampled 0–5 cm layer of the soil did not always solely comprise the most active organic soil layer.

The unexpected large C-accumulation adjacent to our sampling sites at Grillenburg (Grünwald and Bernhofer, 2007) and at the 250-year old deciduous forest at Hainich (Knohl et al., 2003), and the sensational high rate of soil organic C increase in old-growth forests (>400 yr) recently reported from China (Zhou et al., 2006), may be just examples of this soil CO_2-sink. It is otherwise difficult to explain. Old-growth forests are considered to be negligible carbon sinks.

Our results (Fig. 1) indicate that additional NH_4^+-supply may further enforce the sink, also indicated from *in situ* CO_2 concentrations (Fleischer, 2003). Some of the high N-deposition European sites have approached their maximum CO_2-sequestration capacity. This was most pronounced at the forest edge at Grillenburg in June 2005 (Fig. 1). Additional NH_4^+-supply gradually hampered internal soil CO_2-uptake, leading to increased CO_2-emissions. At the highest supply when most of GHR was disclosed (roots excluded in the experiments), the release of CO_2 was almost doubled. A strong CO_2-sink of 49% was indicated. A considerable CO_2-sink had also been initiated at the other European sites, but there the sink could be further enforced after additional NH_4^+-supply, before it gradually weakened.

The mechanisms of the within-soil CO_2-sink remain speculative. Nitrification is, however, not an end in itself, but an energy-yielding reaction enabling use of CO_2 as a carbon source. To generate the energy from nitrification needed for CO_2 reduction to the great extent indicated, N-cycling must be repeated several times. Studies with ^{15}N and isotopic dilution have already shown that this (gross nitrification) is both a general and large-scale process, that can occur with limited nitrate (NO_3^-) leaching (Stark and Hart, 1997; Corre and Lamersdorf,

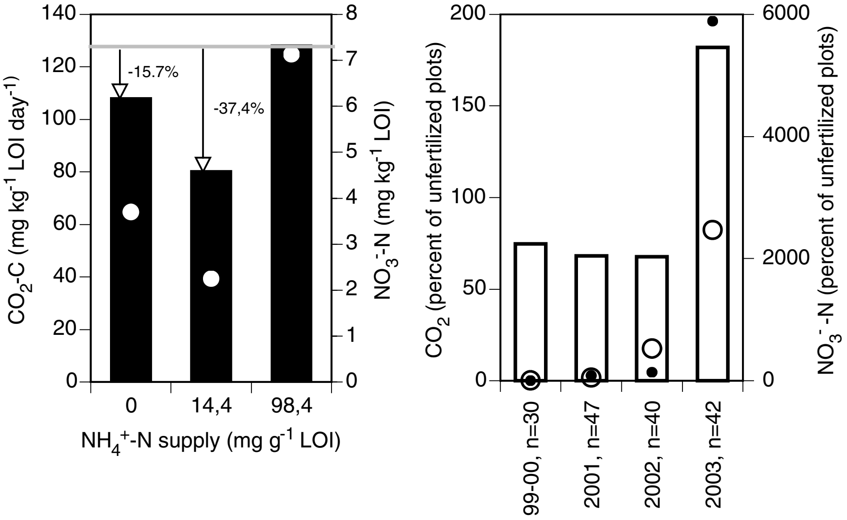

Fig. 2. Average of CO_2–C (bars; n =13) and NO_3^-–N (white dots; n = 10) in the studied soils without, and with additional supply of NH_4^+ to attain CO_2-minimum, and at surplus NH_4^+-additions (left-hand side) and CO_2 in soil atmosphere (bars) and NO_3^-–N in soil water at the same plot (black dots) and all three parallel plots (circles) at the Hjuleberg research Norway spruce forest on former farmland in SW Sweden (right-hand side). Concentrations at the experimentally N-fertilized plots are shown in percent of unfertilized. Black dot 2003 indicates an uncertain but very high value because nitrate at the unfertilized reference was close to zero. n refers to CO_2 sampled all over the frostfree season. Soil water NO_3^- was sampled in May and October–December (2003 in April).

2004), but it is still poorly understood. This situation is most pronounced at the CO_2-minimum where the internal CO_2-sink is strong, concurrently with lowest NO_3^- concentrations despite considerable supply of NH_4^+ (Fig. 2). Nitrifiers are known to develop biofilms (Hagopian and Riley, 1998) and this may explain why the CO_2-consuming subsystem is strongly protected in the soil environment where other inhibiting substances, such as nitrapyrin and potassium ethyl xanthate, also become less influential (Underhill and Prosser, 1987). However, surplus-N-supply finally leads to disintegration of the soil CO_2-sink, and these conditions were also indicated *in situ* at a fertilized, young, Norway spruce forest site on former agricultural land (Table 1; Fig. 2). We suggest that the within-soil CO_2-sink is driven by gross nitrification *sensu strictu*.

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References

Anthoni, P. M, Knohl, A., Rebmann, C., Freibauer, A., Mund, M. and co-authors. 2004. Forest and agricultural land use dependent CO_2 exchange in Thuringia, Germany. *Glob. Change Biol.* **10**, 2005–2019.

Baldocchi, D. and Gu, L. 2003. Multiple ecosystem interactions lead to overall reduced growth in atmospheric CO_2 concentration. *Glob. Change News Lett.* **54**, 23–24.

Corre, M. D. and Lamersdorf, N. P. 2004. Reversal of nitrogen saturation after long-term deposition reduction: impact on soil nitrogen cycling. *Ecology* **85**, 3090–3104.

Fleischer, S. 2003. CO_2 deficit in temperate forest soils receiving high atmospheric N-deposition. *Ambio*. **32**, 2–5.

Galloway, J. N., Dentener, F. J., Capone, D. G., Boyer, E. W., Howarth, R. W. and co-authors. 2004. Nitrogen cycles: past, present and future. *Biogeochemistry* **70**, 153–226.

Gauger, T., Anshelm, F., Schuster, H., Erisman, J. W., Vermeulen, A. T. and co-authors. 2002. Mapping of ecosystem specific long-term trends in deposition loads and concentrations of air pollutants in Germany and their comparison with critical loads and critical levels, Part 1: Deposition Loads 1990–1999. Federal Environmental Agency, Berlin. 207 pp. http://www.nav.uni-stuttgart.de/navigation/forschung/critical_loads/EB_29942210T1.pdf.

Gerstberger, P. (ed.). 2001. Die BITÖK-Untersuchungsflächen im Fichtelgebirge und Steigerwald (in German). Bayreuther Institut für Terrestrische Ökosystemforschung, 194 pp., ISSN 0944-4122.

Grip, H. 2006. *Environmental Effects of Intensive Forest Production Systems* (in Swedish). *Res. Report* 27, Department of Forest Ecology, Umeå, 12pp., ISBN 91-576-7161-3.

Grünwald, T. and Bernhofer, V. 2007. A decade of carbon, water and energy flux measurements of an old spruce forest at the Anchor Station Tharandt. *Tellus* **59B**, 387–396.

Hagopian, D. S. and Riley, J. G. 1998. A closer look at the bacteriology of nitrification. *Agric. Eng.* **18**, 223–244.

Houghton, R. A. 2003. Why are estimates of the terrestrial carbon balance so different? *Glob. Change Biol.* **9**, 500–509.

Knohl, A., Schultze, E.-D., Kolle, O. and Buchmann, N. 2003. Large carbon uptake by an unmanaged 250-year-old deciduous forest in Central Germany. *Agric. Forest Meteorol.* **118**, 151–167.

Mund, M. 2004. Carbon pools of European beech forests (Fagus sylvatica) under different silvicultural management. ISSN 0939-1347, 256 pp.

Nadelhoffer, K. J., Emmet, B. A., Gundersen, P., Kjønas, O. J., Koopmans, C. J. and co-authors. 1999. Nitrogen deposition makes a minor contribution to carbon sequestration in temperate forests. *Nature* **398**, 145–148.

Raich, J. W., Potter, C. S. and Bhagawati, D. 2002. Interannual variability in global soil respiration. 1980–1994. *Glob. Change Biol.* **8**, 800–812.

Rodhe, H., Langner, J., Gallardo, L. and Kjellström, E. 1995. Global scale transport of acidifying pollutants. *Water Air Soil Pollut.* **85**, 37–50.

Spangenberg, A. and Kölling, C. 2004. Nitrogen deposition and nitrate leaching at forest edges exposed to high ammonia emissions in southern Bavaria. *Water Air Soil Pollut.* **152**, 233–255.

Stark, J. M. and Hart, S. C. 1997. High rates of nitrification and nitrate turnover in undisturbed coniferous forests. *Nature* **385**, 61–64.

Underhill, S. E. and Prosser, J. I. 1987. Surface attachment of nitryfying bacteria and their inhibition by potassium ethyl xanthate. *Microb. Ecol.* **14**, 129–139.

Vazquez-Rodriguez, G. A. and Rols, J. L. 1997. Study of the nitrification process with activated sludge: inhibiting effect of ammonia on nitrifying bacteria. *Rev. Sci. Eau.* **10**, 359–375.

Wang, W., Ohse, K., Liu, J., Mo, W. and Oikawa, T. 2005. Contribution of root respiration to soil respiration in a C3/C4 mixed grassland. *J. Biosci.* **30**, 507–514.

Zhou, G., Liu, S., Li, Z., Zhang, D., Tang, X. and co-authors. 2006. Old-growth forests can accumulate carbon in soils. *Science* **314**, 1417.