Scientific report - ESF Exchange Grant 3438

ESF Activity:	**Natural molecular structures as drivers and tracers of terrestrial C fluxes (MOLTER)**
Project title:	**“Effect of nitrogen deposition on composition and turnover of amino sugars in density fractions of forest soils”**
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Host:	**Prof. Dr. Pascal Boeckx, Department of Applied Analytical and Physical Chemistry (ISOFYS), Ghent University, Coupure Links 653, 9000 Ghent, Belgium**
Date of the visit:	**03.10. - 04.12.2011 (9 weeks)**

Introduction

Atmospheric nitrogen (N) deposition increased three- to fivefold over the last century due to human activity, mainly by fossil fuel burning and fertilizer application (Davidson, 2009), which leads to a global distribution of enhanced N emissions also in typically N limited ecosystems like forests. N availability feeds back on carbon (C) dynamics by influencing processes that lead to changes in C fluxes and consequently pool sizes. Since soils represent the largest C pool in terrestrial ecosystems, there is a strong need to understand the underlying processes, because major N-induced changes in soil C storage can affect global C dynamics.

It could be shown that significant N-induced shifts in soil C dynamics corresponded to shifts in microbial community structure, mainly between bacteria and fungi (Compton et al., 2004; Waldrop et al., 2004; Nemergut et al., 2008). Amino sugars are reliable molecular markers to distinct between fungal- and bacterial-derived organic matter (Joergensen & Wichern, 2008). In contrast to phosphor lipid fatty acids, which are labile and represent recently living microbial biomass, amino sugars are effectively stabilized in soil and could be used as a proxy for microbial residues (necromass). Therefore,

amino sugars are a better proxy to investigate medium-to-long-term instead of actual changes in microbial community structure (Glaser et al., 2004).

Although methods for compound-specific stable-isotope-analysis of individual amino sugars exist (Glaser & Gross, 2005; Bodé et al., 2009), only few studies investigated the turnover of amino sugars in soil fractions (Glaser et al., 2006).

Purpose of the visit

The research visit aims at investigating the composition and turnover of amino sugars in forest soil density fractions and testing the hypothesis that increased N deposition affects amino sugar dynamics in soil.

A combined ^{13}C and ^{15}N labeling experiment enables us to distinguish between old (pre-experiment) and new amino sugars (formed during the experiment) and study the influence of N on its turnover. Effects of N on the relative abundance of fungal versus bacterial amino sugars would be indicative for major changes of the soil ecology. Localization of the amino sugars in specific soil fractions would identify soil compartments where important changes occur. This information may also be used to test current hypotheses on stabilization mechanisms of organic matter in soil.

Work during the visit

We used soil samples from a 4-year elevated CO_2 and N-deposition experiment in model forest ecosystems, that were fumigated with ^{13}C-depleted CO_2 and treated with two levels of ^{15}N-labeled fertilizer (Hagedorn et al., 2003). Bulk soil was separated into free light fraction, occluded light fraction and heavy fraction by density fractionation and ultrasonic dispersion. The heavy fraction was further particle-size fractionated with 20 µm as a cut-off (Griepentrog et al., 2011).

Amino sugars were extracted from bulk soil and specific soil fractions. Extracts were analyzed on content and isotopic composition ($\delta^{13}C$) of individual amino sugars using LC-c-IRMS.

Amino sugar extraction

Extraction of amino sugars from bulk soil and soil density fractions was based on the method described by Zhang & Amelung (1996). Therefore, amounts of sample material containing 0.3 mg of N were hydrolyzed by adding 6M HCl (20ml per gram of sample material) and heating at 105°C for 8 hours. Samples were filtered over glass fiber filters (*GF/C, Whatman, Dassel, Germany*) and the filtrate was evaporated to dryness at 40-45°C under reduced pressure to remove HCl. Dried filtrate was redissolved in Milli-Q water (*Direct-Q 3 System, Millipore, Billerica, MA, USA*), transferred in a 2ml tube (*Eppendorf, Hamburg, Germany*) and centrifuged. The supernatant was added onto a cation exchange resin (*AG 50W-X8, Bio-Rad Laboratories, Hercules, CA, USA*). After rinsing the resin with Milli-Q water to remove neutral and negatively charged compounds, the fraction containing amino sugars was eluted with 0.5M HCl and again evaporated to dryness to remove HCl. Dried amino sugars were redissolved in Milli-Q water and transferred in a 2ml tube. After desiccation using a centrifugal vacuum concentrator (*SpeedVac Concentrator, Thermo Scientific, Langenselbold, Germany*), dried amino sugars were stored at -18°C until analysis.

Amino sugar analysis

Compound-specific stable-isotope-analysis of amino sugar extracts was done according to the method described by Bodé et al. (2009). Therefore, we used a high pressure liquid chromatography (HPLC) system existing of an autosampler (*Surveyor Autosampler Plus, Thermo Electron, Bremen, Germany*) and a HPLC pump (*Surveyor MS-Pump Plus, Thermo Electron*) with an analytical anion-exchange column (*PA20 CarboPac, 3 x 150 mm, 6.5 µm*) that was coupled over a wet oxidation interface (*LC Isolink, Thermo Electron*) to an isotope ratio mass spectrometer (IRMS; *DELTA^{PLUS} XP, Thermo Elect*ron).

Results

To study the composition and turnover of microbial residues in forest soils and to investigate the impact of increased N deposition on its dynamics, we extracted amino

sugars from bulk soil and density fractions and determined concentrations and isotope-ratios ($\delta^{13}C$) of individual amino sugars (Glucosamine, GlcN; Galactosamine, GalN, Muramic Acid, MurA) using LC-c-IRMS.

Amino sugar concentrations

In all fractions and bulk soil GlcN-C concentrations are always higher than GalN-C concentrations (Figure 1). From light to heavy fractions both, GlcN-C & GalN-C, are increasing, while the GlcN/GalN ratio is decreasing (Figure 1). This may suggest that GalN is more accumulated relative to GlcN from light to heavy fractions.

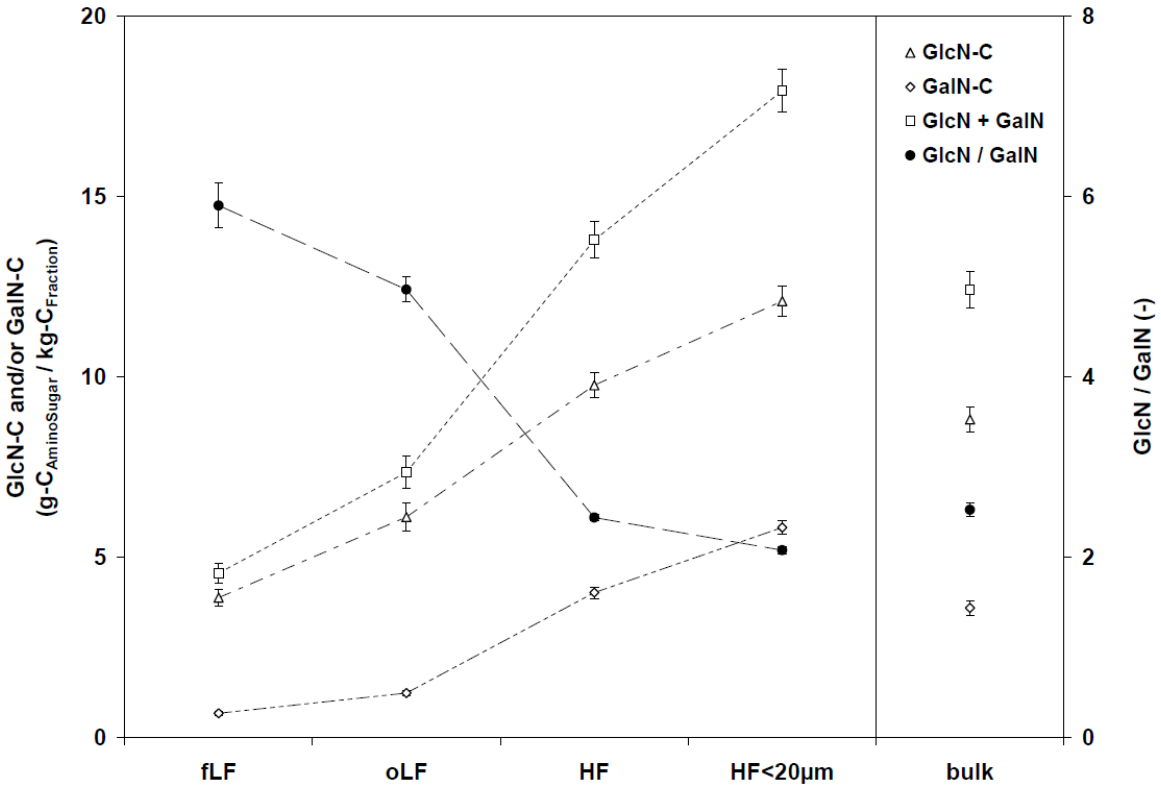

Figure 1: Concentrations of glucosamine (GlcN) and galactosamine (GalN) as well as their sums (GlcN + GalN) and ratios (GlcN / GalN) in bulk soil and density fractions (free light fraction, fLF; occluded light fraction, oLF; heavy fraction, HF; heavy fraction smaller 20 µm, HF<20µm).

Amino sugar isotope ratios

In all fractions and bulk soil GlcN is more enriched in ^{13}C compared to the whole fraction and GalN is even more enriched relative to GlcN (Figure 2). From light to heavy fractions amino sugars, like the whole fraction, are more enriched in ^{13}C (Figure 2). This may indicate that amino sugars are more stabilized in heavy fraction relative to light fraction and that GalN is more stabilized than GlcN.

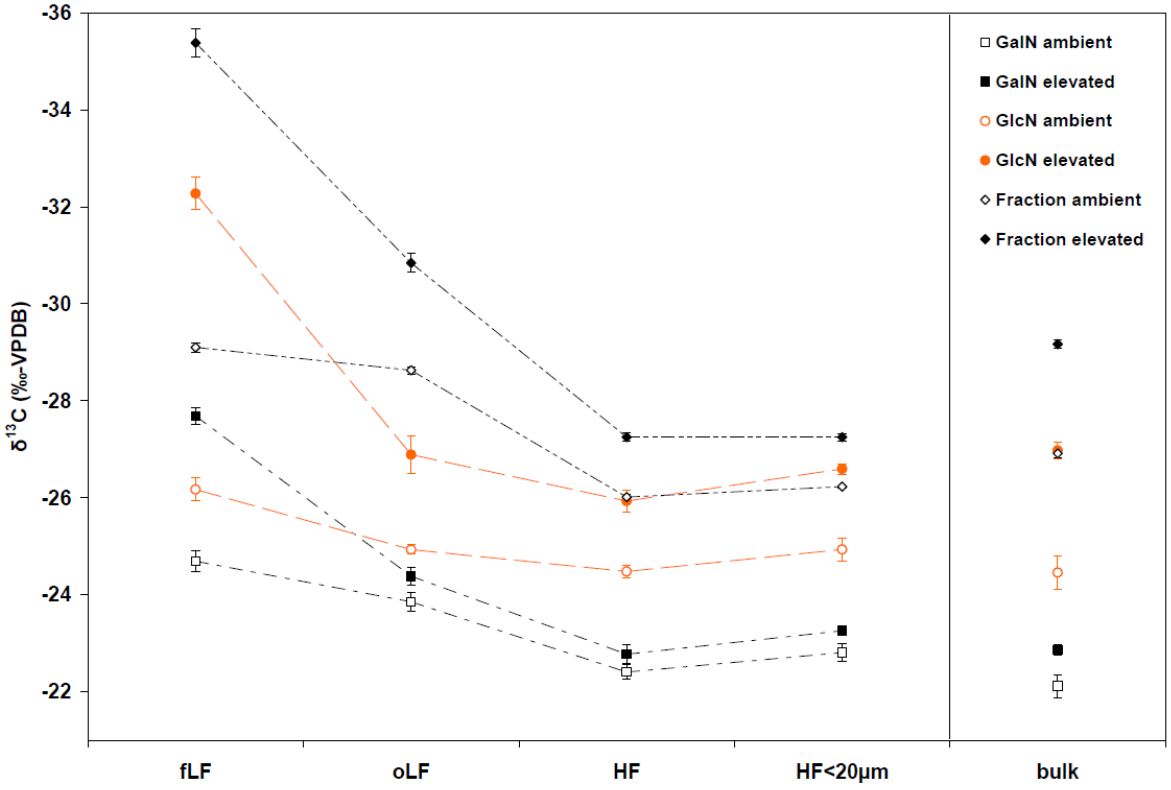

Figure 2: Isotope ratios ($\delta^{13}C$) of glucosamine (GlcN) and galactosamine (GalN) in bulk soil and density fractions (free light fraction, fLF; occluded light fraction, oLF; heavy fraction, HF; heavy fraction smaller 20 µm, HF<20µm) for treatments with ambient and elevated (^{13}C depleted) CO_2.

In all fractions and bulk soil the incorporation of isotopic label (4 years continuous labeling with ^{13}C depleted CO_2) is more pronounced for GlcN than for GalN. Values for GlcN are in the range of whole fraction material. This may suggest a slower turnover of the GalN pool relative to GlcN and whole fraction material.

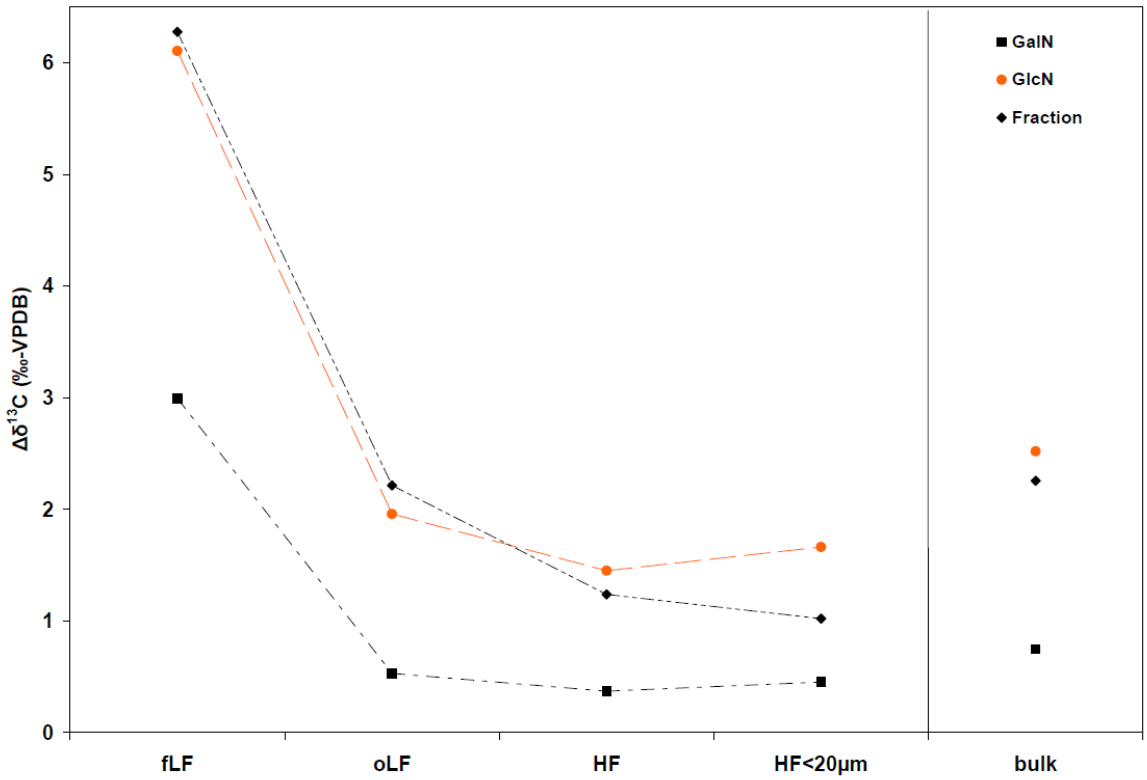

Figure 3: Differences in isotope ratios ($\Delta\delta^{13}C$) of glucosamine (GlcN) and galactosamine (GalN) in bulk soil and density fractions (free light fraction, fLF; occluded light fraction, oLF; heavy fraction, HF; heavy fraction smaller 20 µm, HF<20µm) between treatments with ambient and elevated (^{13}C depleted) CO_2.

Outcomes of the visit

The data shown above as well as further upcoming results of the research visit will be presented at the *General Assembly of the European Geosciences Union (EGU) in Vienna (23.01. – 30.01.2012)* within the session entitled *"Molecular proxies for studying biogeochemical changes in the environment"* and at the *EUROSOIL 2012 in Bari (02.07. – 06.07.2012)* within the session entitled *"Molecular dynamics of soil organic matter: challenges, opportunities and limits"* and a manuscript will be prepared for publication in a peer-reviewed journal (e.g. *Soil Biology & Biochemistry*).

References

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