

1 **Consequence of litter removal on pedogenesis: a case study in Bachs**  
2 **and Irchel (Switzerland)**

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9

10 **Abstract** In forests, soils contain at least twice as much carbon than plants that mostly grow  
11 in the upper layers. Litter at the interface between soils and the atmosphere regulates a  
12 variety of biogeochemical cycles, which are important for both plants and soils and have  
13 possible implications for other environmental components. We have compared leachates  
14 collected during an incubation experiment on: a) beech and oak leaves; b) organic  
15 subhorizons OLn, OLv, OF, and mineral A horizon; c) treated with litter removal (and  
16 untreated) plots, to assess the changes in the chemical composition of the litter layers and  
17 leachates during weathering and their influence on the underlying horizons. . Two different  
18 types of broadleaves –beech and oak– become indistinguishable when they experience  
19 weathering. As a litter horizon is altered, it becomes more stable and loses fewer elements,  
20 both in gaseous and liquid form. The annual removal of litter represents a net loss of biomass  
21 from the system. Nevertheless, the effect on soil in the medium term is not significant.  
22 Leaves and litter horizons were incubated in micro-lysimeters, leached, and characterised  
23 by different analytical approaches, from elementary analyses (dissolved organic carbon,  
24 CO<sub>2</sub> production, nitrogen forms, UV absorptivity) to solid state NMR spectroscopy. The  
25 results reveal that the removal of the litter does not degrade the underlying soils, in direct

1 contrast to what was thought to be the case previously. Moreover, it extends previous  
2 knowledge that litter removal promotes an increase in fulvic acids activity in underlying  
3 horizons. The results demonstrate how this human disturbance, if not combined with other  
4 degradation factors, could promote podzolisation. In a wider outlook, if managed properly  
5 (for example, by burying litter removed after its use in animal husbandry), even the repeated  
6 removal of forest biomass contribute not negatively to the genesis of these soils.

7

8 *Keywords:* Luvisol; Podzol; <sup>13</sup>C CPMAS NMR; SUVA; nitrogen

9

## 10 **1. Introduction**

11 In soil genesis, the alteration products of one individual horizon become reagents within  
12 the next horizon; this phenomenon is particularly evident in the case of podzolisation (Ugolini  
13 et al., 1988). Podzolisation consists of two main chemical components: i) mobile organic  
14 acids, which are the key proton donors that drive the soil processes in the O, E and Bhs  
15 horizons, and govern both soil pH and leaching; ii) these acids dissolve minerals and form  
16 metal–organic complexes that are nested at the Bhs/Bs interface (Ugolini et al., 1977). One  
17 or more plant litter horizons exist above these mineral horizons under natural conditions, at  
18 the interface between the forest plant biomass and soil, and represent one of the potential  
19 key compartments that serves as a C sink (Bellassen and Luysaert, 2014; Janzen, 2004;  
20 Luysaert et al., 2010). Fresh plant litter generally consists of distinguishable vegetal  
21 remains, leaves, needles, roots, bark, twig and wood pieces, either fragmented or whole.  
22 This organic material rapidly or slowly degrades, depending on the local climatic and  
23 biological conditions (Catoni et al. 2016). This thin, delicate layer of organic material can be  
24 easily affected by humans. For instance, forest litter raking as a replacement for straw in  
25 husbandry is an old non-timber practice in forest management that has been widespread in

1 Europe since the seventeenth century (Bürge et al., 2006; Bürge and Gimmi, 2007; Gimmi et  
2 al., 2008). At its peak in 1853, an estimated 50 Tg dry litter per year was raked at the  
3 European level (McGrath et al., 2015). Local historic forest litter-raking results in a long-term  
4 reduction in C pools in soils, which is relevant for C accounting on broader scales (Gimmi et  
5 al., 2013). After long-term raking, it has been calculated that mixed and deciduous forests  
6 show soil carbon depletion by up to 20% of the potential total soil carbon sink without  
7 gathering litter (Gimmi et al., 2013). Several studies have speculated that the influence of  
8 gathering forest litter might also play a key role in soil nutrient biogeochemical cycles  
9 (Glatzel, 1991); Glatzel (1990), Dzwonko and Gawronski (2002), and Vild et al. (2015)  
10 suggested that a progressive depletion of soil nutrients as a consequence of litter removal  
11 occurs .

12 Several ecosystem models enable the impact of anthropogenic activities on ecosystems  
13 to be scaled up (e.g., Kaplan et al., 2012), although the timeframe within which soil carbon  
14 pools can reach equilibrium and/or fully recover remains unclear, as well as the effects on  
15 soil biogeochemical cycles. It is also unclear whether local soil biogeochemical cycles in  
16 individual specific circumstances can be realistically extrapolated, for instance, litter-raking.

17 Human intervention in soil processes has a considerably greater effect than natural  
18 perturbations and thus, exceeds the resiliency of soil to recover to its original condition  
19 (Amundson et al., 2015). Questions include how a soil evolves, whether human intervention  
20 alters one soil horizon and whether the soil formation process becomes slower *sensu*  
21 Simonson (1959) or Runge (1973). The aim of this study was to compare the properties of  
22 two broadleaf litters, to understand whether soil organic matter (SOM) develops and to  
23 develop a framework by which SOM chemistry is altered as it passes through various litter  
24 horizons towards mineral soil. Here, we present results from two beech forests; one mixed  
25 (beech and oak) and a pure forest grown under very similar environmental conditions. The  
26 aim was to understand how species influence the soil upon which they develop, and to

1 evaluate the effect of the periodic removal of the forest litter. We postulate that litters of  
2 different composition, due to the diverse vegetation cover but built over similar soils have  
3 similar properties, and that litter removal, if not combined with other degradation factors,  
4 does not influence soil chemical quality, although can promote pedogenesis towards  
5 podzolisation.

6

## 7 **2. Materials and methods**

### 8 *2.1. Study sites*

9 Litter material was collected from two mature forests in Switzerland. The first stand is at  
10 Irchel (47°32'19"N, 08°36'12"E; elevation 640 m a.s.l.) and is 70 years old and dominated  
11 by *Fagus sylvatica* (L.). The second forest is located in the vicinity of Bachs (47°32'02"N,  
12 08°26'45"E; elevation 589 m a.s.l.). The stand is dominated by *Fagus sylvatica* (L.) with  
13 *Quercus petraea* (Matt.) and some *Pinus sylvestris* (L.) present as a companion species.  
14 The potential natural vegetation is *Luzulo silvaticae*-*Fagetum typicum* (Zimmermann et al.  
15 2006) in both locations.

16 The climate in both stands is characterised by mild winters and moderately warm  
17 summers. Mean January and July air temperatures are 0.3 and 18.6°C, respectively. The  
18 number of days with ground frost is 75 per year and the temperature exceeds 30°C on  
19 average for 4 days per year. The mean annual precipitation is 1,110 mm, of which two-thirds  
20 falls during the growing season, from May to October (Ahrends et al., 2008; Meteo Swiss,  
21 2015; Moser et al., 2010). The parent material is high-lying, consolidated plateau gravel and  
22 conglomerates in Irchel, and moraine in Bachs (Zimmermann et al., 2006). The soils  
23 beneath both stands are OL-OF-A-E/B-Bt-BC+ soils, Haplic Luvisols (Epidystric) (IUSS WG  
24 WRB, 2015) with a dysmoder organic layer with a Humus Index of 7, which is a numerical

1 expression of the humus form along a gradient of increasing litter thickness (Brêthes et al.,  
2 1995; Ponge and Chevalier, 2006).

3 Forest soil organic horizons are normally named on the basis of visual observations  
4 made directly in the field, thus, this is often subjective (Ponge, 1999). Therefore, to obtain a  
5 more objective basis for their classification, two studied stands (Bachs and Irchel) were  
6 compared after a morphological description according to Zanella et al. (2011). A minimum  
7 thickness of horizons for description, diagnosis and sampling purposes has been  
8 established at 3 mm; thinner horizons are considered to be discontinuous. The amount of  
9 organic carbon in dry samples of all litter horizons without living roots was not less than 20%  
10 by mass (ISO 10694:1995 method for carbon elementary analysis). The organic layers here  
11 were sampled according to their morphology. At the Irchel stand, the sequence of horizons  
12 was OLn-OLnv-OLv1-OLv2-OF-meA while at the Bachs stand was OLn-OLv-OFsz-meA  
13 (details in Supplementary Information).

14 We investigated the influence of litter removal in four 100 m<sup>2</sup>-treated plots at each location,  
15 where raking occurs yearly in April as a part of a planned experiment and we sampled a few  
16 days before the annual removal. After raking, within one year a continuous litter layer is  
17 reformed. Control plots of a similar size were established next to these 100 m<sup>2</sup>-treated plots.  
18 We collected leaves from littertraps without soil contact, and from individual litter horizons.  
19 After carefully sampling the whole 1 m<sup>2</sup> of the removed 100 m<sup>2</sup>-layers at both sites, the  
20 uppermost few centimetres of the A-horizon were sampled in both control and treated plots  
21 (five years of annual litter removal). Litter samples were taken at both sites: *Fagus* only at  
22 Irchel, mixed *Fagus* and *Quercus* at Bachs. The leaves, including bud scales, minuscule  
23 branches, seeds and other distinguishable material, collected from littertraps were then  
24 sorted manually in the laboratory. The rationale of this experiment was to compare a mixed  
25 beech litter and a pure beech litter; the collection of leaves via littertraps and their  
26 comparison aimed to determine which properties are species-specific. The collection of

1 individual samples of litter and their comparison aimed to determine which properties were  
2 correlated with depth (i.e., decomposition).

### 3 2.2. *Incubation experiment*

4 The 250 mL-micro-lysimeters (Stericup, Millipore), which allowed the simultaneous  
5 measurement of soil respiration and leaching (Hagedorn and Machwitz, 2007) were  
6 incubated at 20 °C in the dark for 12 weeks (twelve leaching cycles, 4 replicates). After nine  
7 weeks of incubation some of the measured parameters fell below the detection limit or  
8 became constant. Air dried not fragmented litter material ( $4.5 \pm 0.8$  g) was placed into the  
9 filter units that contained an acid-washed glass wool pre-filter (3 g) on top of 0.45  $\mu$ m  
10 Durapore<sup>®</sup> membrane filters. Then, 1.5 g glass wool was placed on top of the soils to allow  
11 a homogeneous distribution of the leaching solution. As we kept constant volume in all  
12 micro-lysimeters, the corresponding weights for the organic layers and A-horizons were in  
13 the range of 2.8–15.2 g, and 3.2–19.8 g, respectively.

14 All the analyses on leachates and emitted gas, including dissolved organic carbon  
15 (DOC), nitrogen forms, cation leaching, UV absorptivity, pH, electrical conductivity (EC) and  
16 CO<sub>2</sub>-production were measured every 7 days while solid-state <sup>13</sup>C CP-MAS spectra on the  
17 solid phases were obtained at the beginning (time 0) and after 12 weeks. At each leaching  
18 cycle, 200 mL of a standard nutrient solution (2.5  $\mu$ M H<sub>3</sub>BO<sub>3</sub>, 400  $\mu$ M CaCl<sub>2</sub>, 100  $\mu$ M  
19 K<sub>2</sub>HPO<sub>4</sub>, 50  $\mu$ M K<sub>2</sub>SO<sub>4</sub>, 0.2  $\mu$ M MnSO<sub>4</sub>, 5  $\mu$ M CuSO<sub>4</sub>, 50  $\mu$ M MgSO<sub>4</sub> und 0.2  $\mu$ M ZnCl<sub>2</sub>) was  
20 applied to the litter with a peristaltic pump for 2 h. The micro-lysimeters were evacuated with  
21 a low suction of 50 hPa using a vacuum-controlled pump (EcoTech). To avoid an uneven  
22 wetting of the litter material, we did not apply suction during the application. Aliquots of the  
23 leachates were stored at 2°C. Soil respiration was measured the following day by placing  
24 the filter units in 1.7-L PE containers, flushing them with CO<sub>2</sub>-free air and measuring the  
25 increase in CO<sub>2</sub> with time. The concentrations of CO<sub>2</sub> were determined by passing the air of

1 the containers through an infrared gas analyser (LI 6252, LI-COR) in a closed cycle for 1  
2 min. The production of CO<sub>2</sub> was calculated by interpolating linearly between two  
3 measurements and integrating over the respective period. As rewetting might stimulate CO<sub>2</sub>  
4 production, this might lead to an overestimation of the total C-mineralisation. However, the  
5 overall mass balance of the 12-week experiment showed a close correlation between  
6 weighed mass loss and the total C losses from the litter as DOC by the weekly leaching and  
7 via respiration. The mean difference between the two independent estimates either based  
8 on C fluxes or on weight loss ranged between -12 to +8% for the different types of samples.  
9 These values, similar to those obtained by Hagedorn and Machwitz (2007) in the same  
10 laboratory, have been validated for the current study.

11

### 12 2.3. *Laboratory analyses*

13 The C and N concentrations in the litter and in the fine earth of the A-horizons were  
14 analysed with a CHN analyser (Model 900, LECO Instruments). The concentrations of DOC  
15 and total nitrogen (TN) in the leachates were measured with a total organic carbon analyser  
16 (TOC-V, Shimadzu). The concentrations of K<sup>+</sup>, Mg<sup>2+</sup>, and Ca<sup>2+</sup> in the leachates were  
17 measured using inductively coupled plasma optical emission spectroscopy (Optima  
18 7300DV, Perkin Elmer), that of ammonium was measured by flow injection analysis (FIAS  
19 300, Perkin Elmer) and that of NO<sub>3</sub><sup>-</sup> with ion chromatography (DX-120, Dionex). Electrical  
20 conductivity (EC) and pH were measured using a pH/conductivity meter (Radiometer). Ionic  
21 strength was estimated as 13·EC expressed in μS cm<sup>-1</sup> (Griffin and Jurinak, 1973; Lindsay,  
22 1979).

23 The molar UV absorptivity of the DOC was measured using a spectrophotometer (Cary  
24 50 UV, Varian). The spectral absorbance of DOC is commonly used to discern a composition  
25 characteristic of dissolved organic matter. Light absorbance by DOC is strongest in the UV

1 region. A range of wavelengths has been related to compositional indicators to discriminate  
2 DOC of differing compositions and we used the measure of carbon normalised (specific) UV  
3 absorbance (SUVA). The  $SUVA_x$  is defined as the UV absorbance at X nm divided by the  
4 DOC concentration ( $\text{mg L}^{-1}$ ) multiplied by 100. Specific UV absorptions normalised to the  
5 DOC concentration (SUVA) have been proposed as indicators of: (i) aromaticity,  $SUVA_{254}$   
6 (Chin et al. 1994; Weishaar et al. 2003; Sanderman et al., 2008), (ii) sparingly degradable  
7 matter  $SUVA_{260}$  (Dunalska et al., 2012), (iii) aliphaticity-aromaticity or the fraction of lignin-  
8 derived 'hydrophobic' DOC,  $SUVA_{285}$  (Dilling and Kaiser, 2002; Rostan and Cellot 1995;  
9 Piirsoo et al., 2012; Stutter et al., 2012; Traina et al., 1990; Hernes et al., 2013), or (iv)  
10 humic-like C,  $SUVA_{335}$  (Cuss and Guéguen, 2013). The SUVA values derived from the UV  
11 absorbance region have been directly related to the aromatic contents associated with the  
12 contributions of humic substances, as determined directly using  $^{13}\text{C}$ -NMR (Weishaar et al.,  
13 2003).

14 Blank values for the standard nutrient solution were: pH = 6.84, the conductivity of the  
15 nutrient solution was  $160.79 \mu\text{S cm}^{-1}$ , TOC was  $277.5 \mu\text{g C L}^{-1}$ , TN was  $15.98 \text{ mg N L}^{-1}$  and  
16 the absorbances were Abs (335 nm) 0.0005, Abs (285 nm) 0.00375, Abs (260 nm) 0.00515,  
17 Abs (254 nm) 0.00605, respectively.

18 Solid-state Nuclear Magnetic Resonance spectra were obtained using a  
19 spectrometer operating at 75.47 MHz (AV300, Bruker). The powdered samples were spun  
20 at 8 kHz in a 4 mm rotor. The spectra were acquired with 1 ms contact time, 4 s recycle time  
21 and 6,000 scans and were processed using 30 Hz line-broadening. The integration area,  
22 expressed as a percentage of the total area, was obtained by splitting the spectrum (Lorenz  
23 et al., 2000). To obtain quantitative results, a preliminary variable contact times experiment  
24 was performed using the following contact time values: 1  $\mu\text{s}$ , 10  $\mu\text{s}$ , 50  $\mu\text{s}$ , 100  $\mu\text{s}$ , 1 ms, 2  
25 ms, 5 ms, 6 ms, 8 ms, 12 ms and 16 ms. To overcome the problem that  $^{13}\text{C}$  CP-MAS is not  
26 usually a quantitative technique, we recorded the variable contact time experiment for one



1 sample to study the different NMR response as a function of the mobility of carbon atoms.  
2 We concluded that all the signals between 0 ppm and 110 ppm can be compared in intensity.  
3 Solid-state  $^{13}\text{C}$  CP-MAS investigation has been carried out by integrating spectra into the  
4 shift regions: 0–45 ppm (alkyl C;  $\text{C}_{14}$ – $\text{C}_{18}$  short chain), 45–60 ppm (N-alkyl C or methoxy;  
5 hydroxy- and epoxy acids), 60–93 ppm (O-alkyl), 93–112 ppm (di-O alkyl), 112–140 ppm  
6 (aromatic), 140–165 ppm phenolic C and 165–190 ppm (carboxyl and amide) (Preston et  
7 al., 1997; Simpson et al., 2008).

8

9

#### 10 2.4. *Calculations and statistics*

11 The size, composition, and material (type of leaves, source horizon) of the collected  
12 samples are specified in Supplementary Information. Twelve samples (3 x 4) were used for  
13 comparison of leaves (Section 3.1), 48 (12 x 4) for comparison of depths (Section 3.2), and  
14 32 (8 x 4) for evaluation of litter removal (Section 3.3).

15 Activity calculations and mass distributions of leachates were performed using the  
16 chemical speciation program MINTEQA2 3.1 (Gustafsson, 2015) with input parameters: ionic  
17 strength, pH, DOC,  $\text{NO}_3^-$ ,  $\text{NH}_4^+$ ,  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ . Temperature was imposed at 25°C,  
18 activity coefficient was estimated according to the Debye-Hückel theory, complexation  
19 model was the Non-Ideal Competitive Adsorption, NICA-Donnan (Milne et al., 2001).

20 All analyses were performed using version 0.8.3-g2dab1e of GNU PSPP software (Free  
21 Software Foundation). To distinguish modifications in leachate parameters, ANOVA and a  
22 post hoc Tukey's test were used (a Kruskal–Wallis test and nonparametric Tukey-type test  
23 were used when the parameters did not conform to a normal distribution and homogeneity  
24 of variance). The whole dataset and the results of the statistical analyses were not presented  
25 due to the large number of data. The arrangement of data for statistical analyses was by

1 time (number of observations per parameter: min 6, max 12), by depth (respectively Bachs  
2 5 and Irchel 6 observations), or by comparison (type of leaves or litter removal treatment).  
3 The number of factors in each comparison was: 2 for comparison of leaves (Section 3.1), 5  
4 for comparison of depths (Section 3.2), and 2 for evaluation of litter removal (Section 3.3).  
5 Paired *t*-tests or Pearson correlation coefficients were calculated in the case of temporal  
6 comparisons only (14/18 degrees of freedom).

7

### 8 **3. Results**

#### 9 *3.1. Comparison of leaves (beech–oak)*

10 The initial pH for beech and oak was above 7. During leaching, the pH dropped to 6.6  
11 in both beech samples and to 5.5 in oak. The initial electrical conductivity was rather site-  
12 specific: EC was 180  $\mu\text{S cm}^{-1}$  at Bachs and 240  $\mu\text{S cm}^{-1}$  at Irchel and decreased to 160  $\mu\text{S}$   
13  $\text{cm}^{-1}$  within a couple of weeks, independently on the site or species (Figure 1, left).

14 During leaching, the released carbon initially appeared to be relatively species-  
15 unspecific but it changed during leaching cycles. Beech, on average, released half of the  
16 DOC compared to that released by oak ( $p < 0.05$ ) during the experimental period (Figure 2,  
17 left). The amount of carbon released to the atmosphere as  $\text{CO}_2$  was species-specific but  
18 changed during leaching cycles. Oak released approximately one sixth less of the  $\text{CO}_2$  that  
19 beeches released ( $p < 0.01$ ).

20 Our SUVA patterns (Figure 3, left) are rather overlaid over time. The  $\text{SUVA}_{254}$  of oak  
21 leaves varied from 2 at the beginning of the leaching period to 4  $\text{L mg}^{-1} \text{m}^{-1}$ . No significant  
22 differences in  $\text{SUVA}_{254}$ ,  $\text{SUVA}_{260}$ , and  $\text{SUVA}_{285}$  were observed and the values for all  
23 collected leachates were extremely similar, and were strictly species-specific. The  $\text{SUVA}_{335}$   
24 values did not change for oak, whereas they peaked after a couple of weeks for beech. In  
25 contrast, the  $\text{SUVA}_{254}$  of beech leaves was significantly ( $p < 0.01$ ) lower than that of oak

1 leaves. The SUVA<sub>254</sub> was strictly species-specific and changed during cycles, reaching a  
2 value close to  $4 \pm 0.2 \text{ L mg m}^{-1}$ . The values of SUVA<sub>254</sub>, SUVA<sub>260</sub>, and SUVA<sub>285</sub> were  
3 correlated by time ( $p < 0.05$ ), while SUVA<sub>260</sub> and SUVA<sub>335</sub> values were un-correlated.

4 Nitrogen concentrations due to leaching were initially site-specific, and then reached  
5 a plateau concentration of  $0.75 \text{ mg N L}^{-1}$  during the leaching cycles. The cumulative TN  
6 release was stable and did not differ between species (Figure 4, left). Changes in the nitrate  
7 concentration was neither species- nor site-specific and nitrates were constantly released  
8 at a concentration of  $0.035 \text{ mg N L}^{-1}$ . The ammonium concentration was species-specific,  
9 with that of oak being lower (2% TN,  $0.07 \text{ mg N L}^{-1}$ ) than that of beech (6% TN,  $0.20 \text{ mg N}$   
10  $\text{L}^{-1}$ ) ( $p < 0.5$ ). During leaching, the Ca concentration increased, whereas that of Mg was  
11 stable and that of K decreased. Cumulatively, Ca and K release appeared to be more site-  
12 specific than species-specific, although not significantly. The Mg and ammonium  
13 concentration was similar for all leaves (Figure 5, left).

14 The resonance peaks were integrated according to the above-reported regions and the  
15 relative intensities are summarised in Table 1. After 12 weeks of leaching, the amount of  
16 surface waxes (alkyl signals at 0–45 ppm) and the major components of the leaf cuticle and  
17 short-chain ( $\text{C}_{14}$ – $\text{C}_{18}$ ) hydroxy- and epoxy acids decreased in beech, whereas  
18 carbohydrates increased. No differences between oak and beech species (Figure 6a) and  
19 no site-specific differences for beech leaves were observed (Figure 6b). The amount of  
20 tannins and lignin increased in beech, but decreased in oak; the alkyl/O-alkyl (0–45 ppm/60–  
21 93 ppm) ratio decreased significantly after incubation in both species, due to the lower  
22 amounts of alkyl components (Table 1).

23

### 3.2. Depth comparison

During leaching, the pH dropped by one unit on average, the mean difference between leaves and the first mineral horizons was two pH units (Figure 1, middle). Electrical conductivity was relatively stable in the surface horizons, but increased dramatically after one month of leaching in deeper horizons.

The amount of carbon released during leaching was initially one-quarter of the carbon mineralised as CO<sub>2</sub> on average (Figure 2, middle). Although no statistically significant differences with time were observed in individual litter horizons in terms of DOC release, sub-surficial litter horizons mineralised more carbon than lower horizons. No significant differences in the SUVA<sub>254</sub>, SUVA<sub>260</sub>, and SUVA<sub>285</sub> were observed and all leachates collected showed extremely similar values that were strictly correlated ( $r > 0.98^{**}$ ). Although not significantly with depth, the values increased from the bottom throughout the profile and during cycles, reaching a common value close to  $5 \pm 0.5 \text{ L mg m}^{-1}$  (Figure 3, middle). The SUVA<sub>335</sub> showed no significant trend, either in depth or time.

After leaching, the amount of alkyl-C groups increased in both OF horizons, although not homogeneously (Table 1; Figure 7a and 7b). The amount of N-alkyl-C groups, including lignin, (45–60 ppm) also increased, but to a similar degree at Bachs and Irchel. At the same time, the concentration of plant carbohydrates and their degradation products, which were in the ranges of 60–93 ppm and 93–112 ppm, respectively (Baldock et al., 1992), decreased markedly and similarly in both OF horizons. Although the composition of the A mineral horizons in Bachs and Irchel was similar, different NMR spectra were observed between two overlying OF litter horizons (Figure 7c), where the alkyl/O-alkyl ratios increased after leaching. This is because the concentration of alkyl- and methoxyl-C increased (respectively >15% and >5%), whereas that of O-alkyl and di-O-alkyl-C decreased (>15%).

1 The nitrogen concentration was relatively constant for leaves and the upper horizons,  
2 with a mean of 0.96 mg N L<sup>-1</sup> throughout the whole leaching period. The TN increased  
3 considerably in the lower horizons and the highest release occurred in the A mineral  
4 horizons (Figure 4, middle). The Mg concentration remained relatively constant during the  
5 leaching cycles (0.2 ± 0.03 mM), whereas that of Ca increased and that of K decreased,  
6 reaching the same value (0.5 ± 0.08 mM), independently of the litter horizon. Cumulatively,  
7 Ca release from beech leaves in the mineral A horizons was significantly higher than that of  
8 the OF horizons ( $p < 0.01$ ); K release from beech leaves in the mineral A horizons was  
9 significantly ( $p < 0.01$ ) higher than that from the OLv and OF horizons and the Mg release  
10 in beech leaves and mineral A horizons was not significantly different. Ammonium was  
11 released prevalently from the mineral A and lower litter horizons significantly ( $p < 0.01$ ) more  
12 than from beech leaves and the OLn horizons (Figure 5, middle). The intermediate litter  
13 horizons that were sandwiched between freshly deposited leaves and mineral soil horizons  
14 release less cations, indirectly demonstrating their inability to retain them.

15

### 16 3.3. *Influence of annual litter removal*

17 During leaching, the pH increased in the mineral horizons, but decreased in the litter  
18 horizons, reaching a mean value of 6.5 after one month (Figure 1, right), after which the pH  
19 in the mineral horizons decreased (less in treated plots than in control plots) and that of the  
20 litter horizons increased (without any significant difference between the treated and control  
21 plots). The electrical conductivity was relatively stable in the first litter horizons (170 μS cm<sup>-1</sup>),  
22 increasing dramatically after one month of leaching in the litter horizons, but no significant  
23 difference between control and treatment plots was observed.

24 The annual removal of litter results in a lower mineralisation as CO<sub>2</sub> and less leaching of  
25 DOC from the treated plots compared to control plots, both in the mineral and the first litter

1 horizons (Figure 2, right). No significant differences in the SUVA<sub>254</sub>, SUVA<sub>260</sub>, and SUVA<sub>285</sub>  
2 were observed and all leachates collected showed similar and correlated values with time ( $r$   
3  $> 0.65^*$ ). The SUVA<sub>335</sub> peaked after three weeks of leaching, and was significantly lower in  
4 the treated plots than in the control plots (Figure 3, right). Due to the small differences in the  
5 C content of the litter samples, no conclusions were possible from the NMR results. The  
6 nitrogen concentration increased in the mineral horizons, but was relatively constant for the  
7 litter horizons, at the end of the leaching periods and had a mean of 9.2 and 2.4 mg N L<sup>-1</sup>,  
8 respectively. The greatest release occurred in the control plots compared to the treated  
9 plots, although the difference was not significant (Figure 4, right). The release of K<sup>+</sup>, Ca<sup>2+</sup>,  
10 Mg<sup>2+</sup> and NH<sub>4</sub><sup>+</sup> was always lower in the treated horizons than in the control plots, although  
11 not significantly (Figure 5, right).

12

#### 13 **4. Discussion**

14 To compare two similar litters, consisting of pure beech and beech mixed with oak, we  
15 identified the differences independently. The small initial differences were highlighted via  
16 leaching: oak leaves produced a more acidic leachate (pH 5.5) than beech (pH 6.5),  
17 although both had relatively similar ionic strengths. We did not measure significant  
18 differences in the composition of beech and oak leaves, apart from relatively more cutins  
19 and waxes in oak leaves (Table 1, Figure 6a). The leaching homogenised the  
20 characteristics. Leaching of DOC represents an insignificant loss of C from soils, but a major  
21 input to ground and surface water. Climate and the type of tree species are the most  
22 important factors that regulate site-to-site variation in DOC concentrations and fluxes in  
23 temperate forests (Borken et al., 2011). In broadleaved forests, Hansen et al. (2009) found  
24 no significant differences between species in foliar or total nutrient fluxes, but nutrient fluxes  
25 were higher in oak and beech. The amount of methoxyl C groups, which had a concentration

1 of 45–60 ppm, increased in both species, whereas the alkyl/O-alkyl ratio decreased (Table  
2 1). Although alkyl C and O-alkyl C are both labile, easily degradable components (Albrecht  
3 et al., 2015), O-alkyl C (holocellulose) was lost during the initial decomposition phase of litter  
4 (Preston et al., 1994), whereas under these climatic conditions alkyl C (cutins and waxes)  
5 and aromatic C and phenolic C (lignin and condensed tannins) accumulated (Kögel-  
6 Knabner, 2002; Lemma et al., 2007; Meentemeyer, 1978). The same aromatic-C content in  
7 DOC, as estimated by the  $SUVA_{254}$ , was observed in beech and oak litter by Jaffrain et al.  
8 (2007). The  $SUVA_{254}$  of oak leaves from 2 at the beginning of the leaching period to 4 L  
9  $mg^{-1} m^{-1}$  showed a similar pattern to that described in other studies (Chow et al., 2011).  
10 Rates of C turnover of beech and oak litter have been shown to be similar in other studies  
11 at higher latitudes, as well as concentrations of N, K, Ca and Mg (Vesterdal et al., 2012).

12 Strobel et al. (2001) reported few differences in the soluble C originating from different  
13 species while here, the two species could be distinguished by C loss: oak lost more carbon  
14 than beech. Oak transformed comparable amounts of carbon into  $CO_2$  and DOC, whereas  
15 beech mineralised much more C (about three times more) than it converted into DOC. In  
16 general, DOC in the leachates was more aromatic in oak and more aliphatic in beech (Figure  
17 3, Figure 6a), but in the course of leaching, this became homogeneous and indistinguishable  
18 between the species. The amount of total N did not differ between species, but K was  
19 increasingly concentrated in the beech leachates, whereas Ca accumulated in oak. Oak  
20 species produce changes in soil nutrients, particularly N and Ca, affecting the soil microbial  
21 community, in terms of size and composition (Aponte et al., 2013).

22 Plants are an important factor in the acidification of soils through several pathways.  
23 These include the leaching of organic acids from litter and imbalances in the ion uptake by  
24 plants (Ehrenfeld et al., 2005). Augusto et al. (2002) grouped tree species in the order of  
25 decreasing acidifying ability: 1 (*Picea* and *Pinus*) > 2 (*Abies* and *Pseudotsuga*) > 3 (*Betula*,  
26 *Fagus* and *Quercus*) > 4 (*Acer*, *Carpinus*, *Fraxinus*, and *Tilia*). It is generally presumed that

1 the pathways of soil acidification involve feedbacks but species-related pH patterns are  
2 attenuated with depth: the differences among species prevail in the forest floors, less evident  
3 respectively in the first soil horizon and in sub-soil horizons (Finzi et al., 1998). The pH ( $t =$   
4  $5.09^{***}$ ), the K:Ca ratio ( $t = 4.52^{***}$ ) and the ratio between C mineralised as CO<sub>2</sub> and  
5 solubilised as DOC ( $t = 6.24^{***}$ ) were higher in beech, whereas the C:N ratio ( $t = 12.23^{***}$ )  
6 was higher in oak. The two deciduous leaves, beech and oak showed no statistically  
7 significant differences measured in leachates for most of the measured parameters,  
8 including the optical properties of dissolved organic carbon. The C:N ratio was lower than  
9 that reported in other studies (Cools et al., 2014). Litters with low C:N ratios, (low ratios of  
10 N:lignin or low concentrations of polyphenols) decompose more rapidly and completely  
11 whereas litters with the opposite qualities tend to decompose slowly; these patterns are then  
12 paralleled by vegetation patterns (Ehrenfeld et al., 2005; Lavorel and Garnier, 2002).

13 In plant tissues, K is only electrostatically bound to membranes, and is thus rapidly  
14 leached when these are destroyed; Ca is essentially structurally bound and is usually less  
15 soluble than Mg (Joergensen and Meyer, 1990), which is not what was observed for the  
16 leaves here. Nevertheless the concentrations of Ca, Mg and K were similar to those  
17 described elsewhere (e.g., Labaz et al., 2014). Similar findings have been described by  
18 other authors (Joergensen and Meyer, 1990; Hristovski et al., 2014), such as an increased  
19 Ca concentration in beech as a linear function vs. accumulated mass loss. Here, the  
20 concentration of K was correlated with the pH<sup>\*\*\*</sup> and was negatively correlated with the  
21 concentration of Ca<sup>\*\*</sup> and Mg<sup>\*</sup>. The Ca and Mg<sup>\*\*</sup> concentrations were correlated in both  
22 leaves and sites. The paired correlations of beech patterns show this strong relationship  
23 between pH and Ca<sup>\*\*</sup> or K<sup>\*\*\*</sup>.

24 When leaves fall to the ground, they begin to interact with the soil ecosystem. Leaves  
25 decompose in the litter and together with other (organo-)mineral components, release more  
26 ions, but also buffer the pH changes during decomposition (Figure 1, middle). Freshly



1 senesced litter produces DOC, which can be leached from the soil profile, respired, or  
2 contributes to soil organic matter accumulation. According to some authors, organic matter  
3 originating from leaf litter contributes more to respiration (e.g., Uselman et al., 2012). When  
4 leaves are transformed into litter organic horizons, less carbon is lost. In general, much more  
5 C is respired than is lost through leaching. The deeper litter horizon OF loses less C than all  
6 other horizons, including the A mineral horizon. The quality of DOC is initially very different  
7 between individual litter horizons, but is homogenised during weathering (Figure 3, middle),  
8 indicating that a potentially rapid pedogenesis of these organic horizons occurs. The first  
9 surface horizons release more than half of the nitrogen compared to deeper horizons ( $p <$   
10  $0.001$ ) (Figure 8). Over time, N is released from deeper horizons; the concentration of nitrate  
11 and ammonium exceeds  $1 \text{ mg L}^{-1}$ . The C:N ratio of the beech leaves is always higher than  
12 that in the first horizon of litter, and exceeds 25. Compared to in the leaves and the first OLn  
13 horizon, the lower horizons show a ratio below 10. Carbon is sequestered in soil when a low  
14 soil C:N ratio promotes microbial C-use efficiency, new SOM formation and stabilisation  
15 (Alberti et al., 2015). Unaltered leaves and the A horizon leach more K and Ca compared to  
16 all other horizons, whereas OF, the more altered organic horizon, leaches fewer cations  
17 than all the other horizons. Labile organic fractions, for example sugars, are typically not  
18 highly concentrated in solution as they are initially produced by fresh litter as an energy  
19 source for microbial activity (Uselman et al., 2012). After leaching (Table 1, Figure 7a, Figure  
20 7b), both OF horizons showed an increase in the alkyl/O-alkyl ratio as a consequence of a  
21 decrease in the amount of alkyl C, carbohydrates, hemicellulose and cellulose. We observed  
22 no increase in aromatic C at Irchel (pure beech), in contrast to an increase at Bachs, where  
23 beech leaves were mixed with a small quantity of oak leaves. These findings agree with  
24 those reported by Quideau et al. (2005). In temperate forests, humus forms are more  
25 affected by soils, pedoclimatic conditions and climate than by forest canopies (Ponge et al.,

1 2011; Ponge, 2013), which is demonstrated by the results here by the comparison of two  
2 beech stands.

3 Intuitively, the removal of forest litter should have consequences, including negative  
4 effects on soil biogeochemical cycles (Bürge, 1999; Dzwonko and Gawronski, 2002). The  
5 evidence, however, is variable; in some cases, the collection of forest litter was shown to  
6 have no significant consequences (Mariani et al., 2006), but a positive feedback effect on  
7 soil fertility was also demonstrated (Matsushima et al., 2014). The annual removal of litter  
8 lowers the value of many parameters in the case of mineral A horizons, except for the pH,  
9 which is unaffected, or the Ca:K ratio, which is reversed during leaching. Total nitrogen and  
10 cations were less concentrated in the leachates of the A horizons following litter removal.  
11 The first disturbed litter horizons are removed annually but actually are not very different  
12 from those in undisturbed conditions that evolve on other organic horizons and not directly  
13 in contact with the mineral A horizons. The only anomalies are the SUVA<sub>335</sub> and the  
14 concentration of ammonium. Collecting and removing biomass removes carbon from the  
15 system, so that as a result of raking, less carbon is lost by treated litter horizons during  
16 leaching, due to their lower initial total amount. In addition to differences in the initial C  
17 content, differences also exist in the organic matter quality. Similar findings have also been  
18 reported by Fuentes et al. (2014), although their study concluded that the SUVA<sub>254</sub> was not  
19 affected by litter removal. The only exception is humic-like C (as inferred by the SUVA<sub>335</sub>),  
20 which was significantly less abundant in the plots where litter was removed annually.

21 Modelling the liquid phase by estimating the speciation of ions in leachates, we observed  
22 the greatest changes in the OLn and OLv horizons, where dissolved carboxylic fulvic acids  
23 (FAs) exceed phenolic FAs in the calculated equilibrated mass distribution (Figure 9). In  
24 particular, modelled carboxylic groups on FAs increase both as a consequence of leaching  
25 and as a result of the annual removal of litter. When a cation, in particular Ca, is bound to  
26 SOM, it is not lost as a result of litter removal, and the A mineral horizon is also not affected.

1 So, the mobile organic acids, which are the key proton donors that drive the processes of  
2 upper horizons, can become reagents within the next horizon governing both pH and  
3 leaching, in other words promoting podzolisation. These mechanisms are well known  
4 occurring under coniferous species that *per se* promote podzolisation (Augusto et al., 2002;  
5 Miles, 1985; Nielsen et al., 1999; Sohet et al., 1988) while were not documented yet under  
6 deciduous species. Deciduous species in fact grow up only after soil diverged from podzolic  
7 soils had taken place (Willis et al., 1997). Here litter raking becomes one of the triggering  
8 mechanisms responsible for this type pedogenesis.

9 Many opinions have been proposed regarding how trees affect soils. Plant–soil reactions  
10 arise each time plants provoke species-specific modifications to soil properties that in turn  
11 affect the growth of the plants (Bever, 1994; Ehrenfeld et al., 2005). Plant–soil feedbacks  
12 impact on species coexistence (Brandt et al., 2013; van der Putten et al., 2013) so changes  
13 in tree species may lead to the changes in soil conditions (Augusto et al. 2002; Finzi et al.,  
14 1998; Willis et al., 1997) altering soil fertility through different litter quality (Aponte et al.,  
15 2013). In fact, litterfall mass and quality commonly differs for different species growing on  
16 the same site (Binkley and Giardina, 1998). Leachates from different litters are important to  
17 weathering processes, soil pH, microbial community composition and nutrient cycling  
18 (McDowell and Likens, 1988; Qualls et al., 1991), but there have been few suggestions in  
19 the Literature that the leached dissolved organic carbon creates feedbacks. The main issue  
20 is that the key differences in terms of plant-soil feedback between species have been  
21 demonstrated between conifers and deciduous, while within the individual group (coniferous  
22 or deciduous) the differences are difficult to isolate. Our results confirm that although the  
23 litter of the two species differ enough (e.g. oak leaves produced a more acidic leachate than  
24 beech) their final impact on similar soils does not discriminate them detectably. This confirms  
25 that although the stock of ions would increase under coniferous compared to deciduous  
26 species, such as *Fagus* or *Quercus* (Augusto et al., 2002; Bonneau et al., 1979), both of

1 them have a plant–soil feedback balance close to equilibrium (Bauzon et al., 1969). The  
2 interface between litter and mineral horizons, in the long-term, remains a challenging task  
3 for further studies on the mechanism-specific of stabilized OM (Kaiser et al., 2016), including  
4 morphometric approaches (Bryk, 2016).

5  
6

## 7 **Conclusions**

8 We investigated two broadleaved litters; beech and oak, and observed some differences  
9 in carbon losses during decomposition, in leachates in particular. However, the initial  
10 dissimilarities between the two leaves became homogenised over time and the litters  
11 became indistinguishable. When leaves fall on the soil ecosystem, they become litter and  
12 alter rapidly. As a litter horizon is altered, it becomes more stable and loses fewer elements,  
13 in gaseous or liquid form. In these stands, similar soils affect litter more than different leaves  
14 influence litter.

15 The annual removal of litter represents a net loss of biomass from the system.  
16 Nevertheless, the effect on soil in the medium term is not relevant. However, an increase in  
17 the concentration of small fulvic molecules is easily absorbed by the rhizosphere, which is  
18 less resistant to microbial activity. Therefore, more microbial activity causes more CO<sub>2</sub> to be  
19 released, but nutrients become more available. This human disturbance could promote  
20 pedogenesis towards podzolisation.

21

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4

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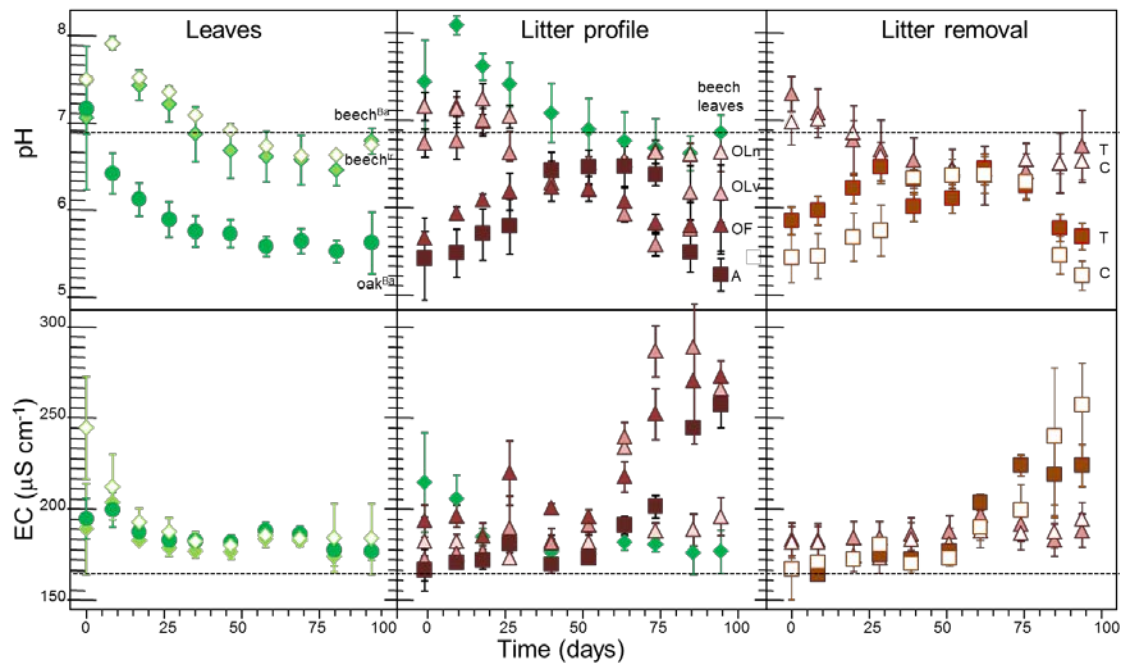
1 **Table 1.** Relative intensities (percent of total area) of the <sup>13</sup>C CP-MAS. Samples before  
 2 leaching, in italics, and after leaching, in bold.

3

Type	Site	0- 45 ppm	45- 60 ppm	60- 93 ppm	93- 112 ppm	112- 140 ppm	140- 165 ppm	165- 190 ppm	Alkyl/ O- Alkyl	
leaves	<i>beech</i>	<i>Irchel</i>	19.4	4.6	51.4	11.3	7.5	3.6	2.1	0.38
		<b>Irchel</b>	17.6	6.2	51.9	10.6	4.9	4.4	4.4	0.34
		<i>Bachs</i>	18.3	4.3	52.6	11.7	7.2	3.7	2.2	0.35
	<i>oak</i>	<b>Bachs</b>	15.1	6.9	50.0	12.4	8.4	3.9	3.2	0.30
		<i>Bachs</i>	23.0	3.7	46.2	9.9	7.6	4.6	4.3	0.50
		<b>Bachs</b>	21.9	8.3	46.9	10.0	7.1	2.8	2.8	0.47
OF	<i>Bachs</i> <sup>§</sup>	20.6	7.7	48.4	10.7	7.2	2.2	2.5	0.43	
	<b>Bachs</b> <sup>§</sup>	23.8	8.1	38.0	9.0	10.7	5.5	4.9	0.63	
	<i>Irchel</i>	19.7	6.8	46.8	11.0	8.1	4.1	3.5	0.42	
	<b>Irchel</b>	28.0	7.3	40.1	8.8	8.1	3.9	3.6	0.70	

4 <sup>§</sup>OFsz

1 **Figure 1.** Electrical conductivity (below) and pH (above) through the 12 weeks period of  
 2 leaching. Symbols indicate means while whiskers indicate standard deviations. Dotted lines  
 3 indicate respectively pH and EC of the inlet leaching solution.  
 4 Leaves comparison: diamonds indicate beech leaves (beech<sup>lr</sup> and beech<sup>Ba</sup>) while circles  
 5 indicate oak leaves (oak<sup>Ba</sup>); open symbols indicate Irchel while filled symbols indicate Bachs  
 6 stand respectively [four replicates].  
 7 Litter profile: diamonds indicate beech leaves, squares indicate the first mineral A horizons,  
 8 while triangles indicate individual litter horizons (the progressively paler colors indicate the  
 9 progressively more surficial horizons) [eight replicates (4 replicates • 2 sites)].  
 10 Litter removal: triangles indicate first litter horizons while squares indicate first mineral  
 11 horizons (open symbols indicate control plots) [eight replicates (4 replicates • 2 sites)].



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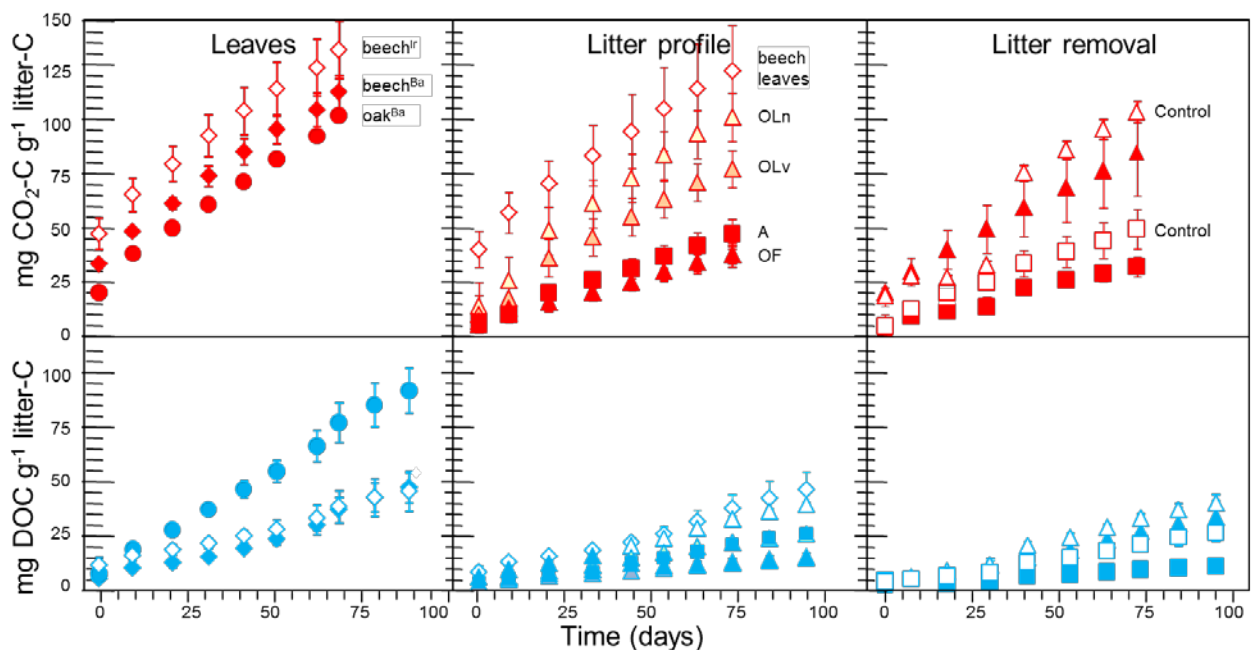
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1 **Figure 2.** Cumulative C mineralization as CO<sub>2</sub> (red symbols) and DOC release (blue symbols) through the 12 weeks period of leaching (9 weeks only are shown in the case of  
 2 symbols) through the 12 weeks period of leaching (9 weeks only are shown in the case of  
 3 CO<sub>2</sub> as the further increases were below the detection limit). Symbols indicate means while  
 4 whiskers indicate standard deviations.

5 Leaves comparison: diamonds indicate beech leaves (beech<sup>Ir</sup> and beech<sup>Ba</sup>) while circles  
 6 indicate oak leaves (oak<sup>Ba</sup>); open symbols indicate Irchel while filled symbols indicate Bachs  
 7 stand respectively [four replicates].

8 Litter profile: diamonds indicate beech leaves, squares indicate the first mineral A horizons,  
 9 while triangles indicate individual litter horizons (the progressively paler colors indicate the  
 10 progressively more surficial horizons) [eight replicates (4 replicates • 2 sites)].

11 Litter removal: triangles indicate first litter horizons while squares indicate first mineral  
 12 horizons (open symbols indicate control plots) [eight replicates (4 replicates • 2 sites)].



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1 **Figure 3.** Temporal trends of specific ultraviolet absorbances ( $SUVA_x$ ) of leachates.

2 Symbols indicate means while whiskers indicate standard deviations.

3 Leaves comparison: diamonds indicate beech leaves (beech<sup>lr</sup> and beech<sup>Ba</sup>) while circles

4 indicate oak leaves (oak<sup>Ba</sup>); open symbols indicate Irchel while filled symbols indicate Bachs

5 stand respectively [four replicates].

6 Litter profile: green diamonds indicate beech leaves, brown squares indicate the first mineral

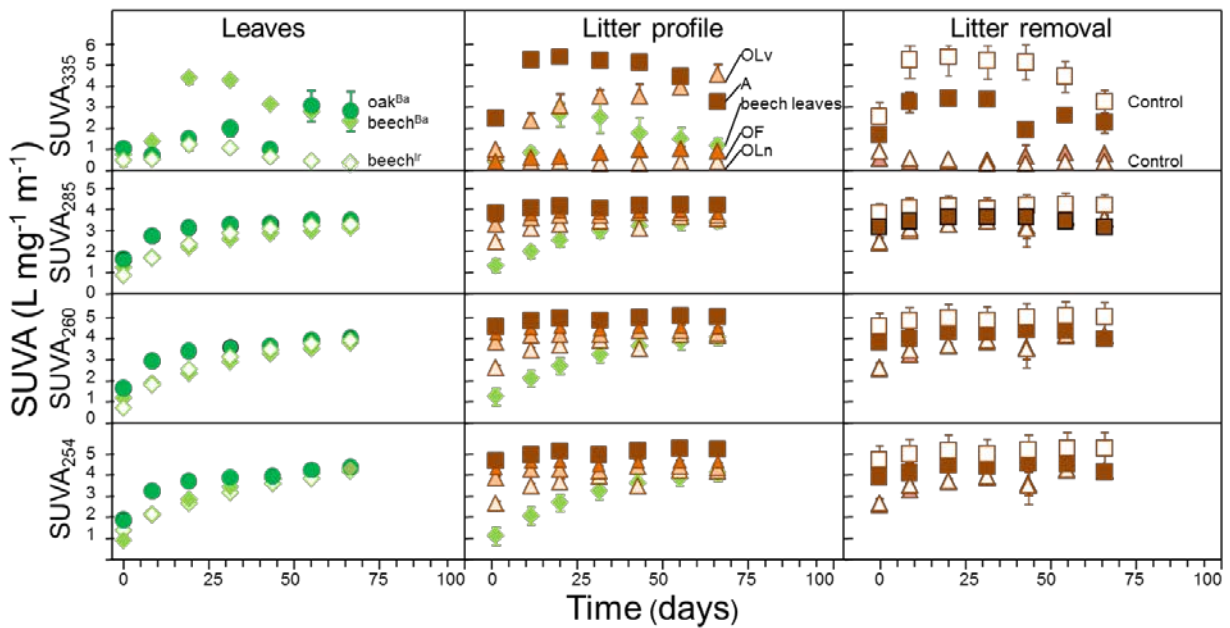
7 A horizons, while triangles indicate individual litter horizons (the progressively paler colors

8 indicate the progressively more surficial horizons) [eight replicates (4 replicates • 2 sites)].

9 Litter removal: brown triangles indicate first litter horizons while brown squares indicate first

10 mineral horizons (open symbols indicate control plots) [eight replicates (4 replicates • 2

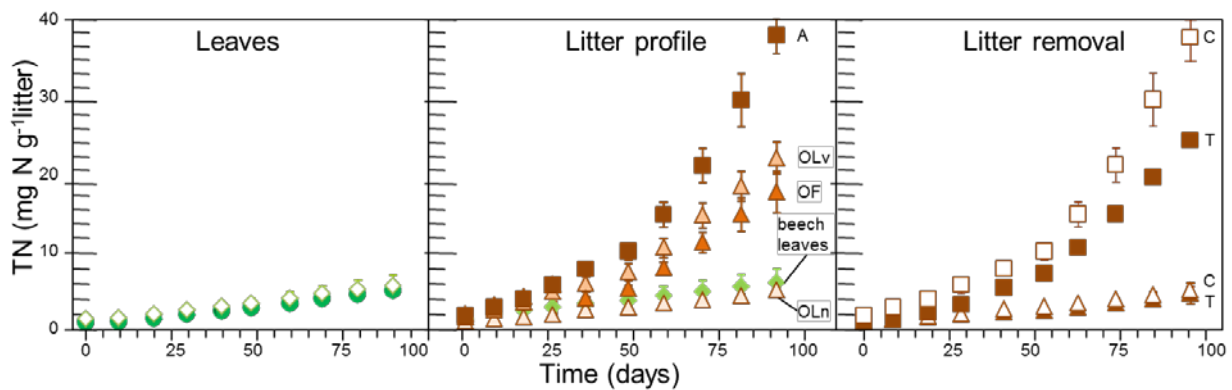
11 sites)].



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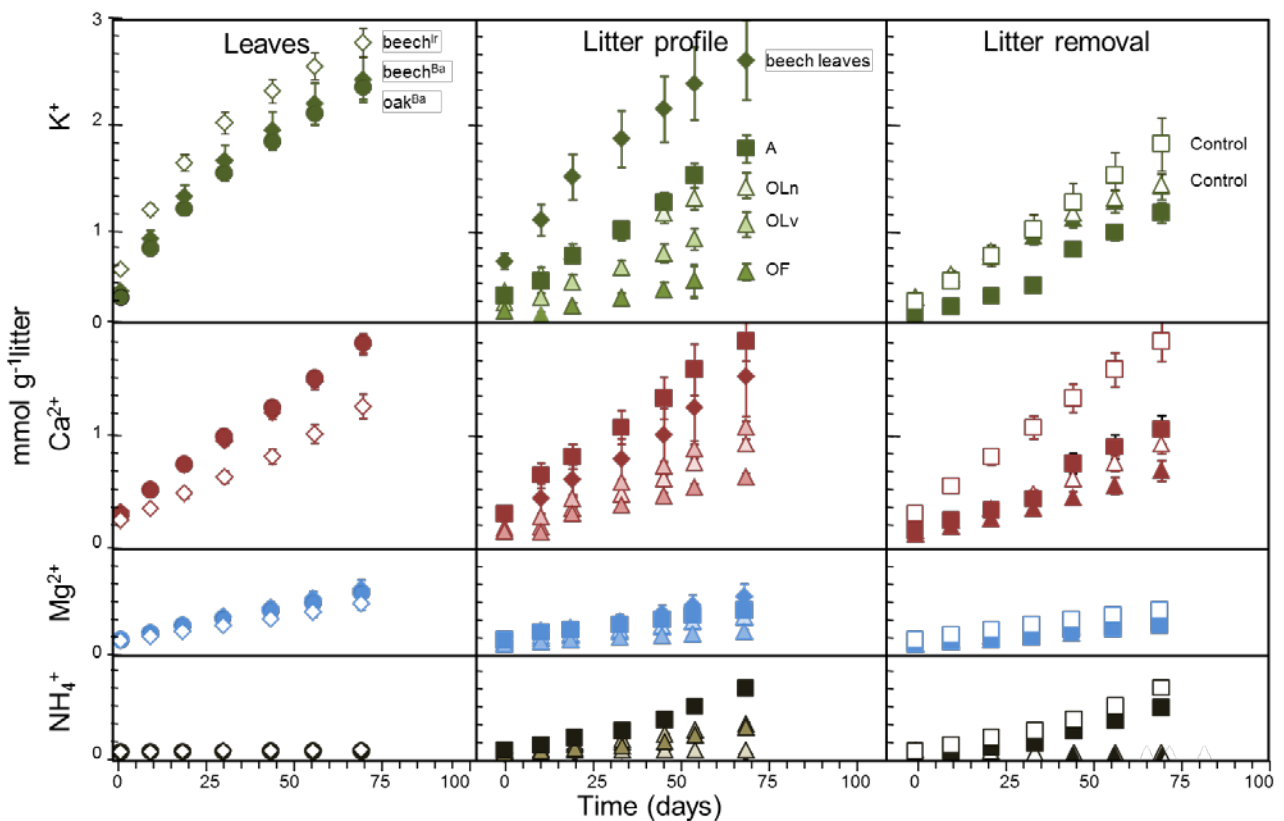
1 **Figure 4.** Cumulative TN release through the 12 weeks period of leaching. Symbols indicate  
 2 means while whiskers indicate standard deviations.  
 3 Leaves comparison: green diamonds indicate beech leaves while circles indicate oak  
 4 leaves; open symbols indicate Irchel while filled symbols indicate Bachs stand respectively  
 5 [four replicates].  
 6 Litter profile: green diamonds indicate beech leaves, brown squares indicate the first mineral  
 7 A horizons, while triangles indicate individual litter horizons (the progressively paler colors  
 8 indicate the progressively more surficial horizons) [eight replicates (4 replicates • 2 sites)].  
 9 Litter removal: brown triangles indicate first litter horizons while brown squares indicate first  
 10 mineral horizons (open symbols indicate control plots) [eight replicates (4 replicates • 2  
 11 sites)].



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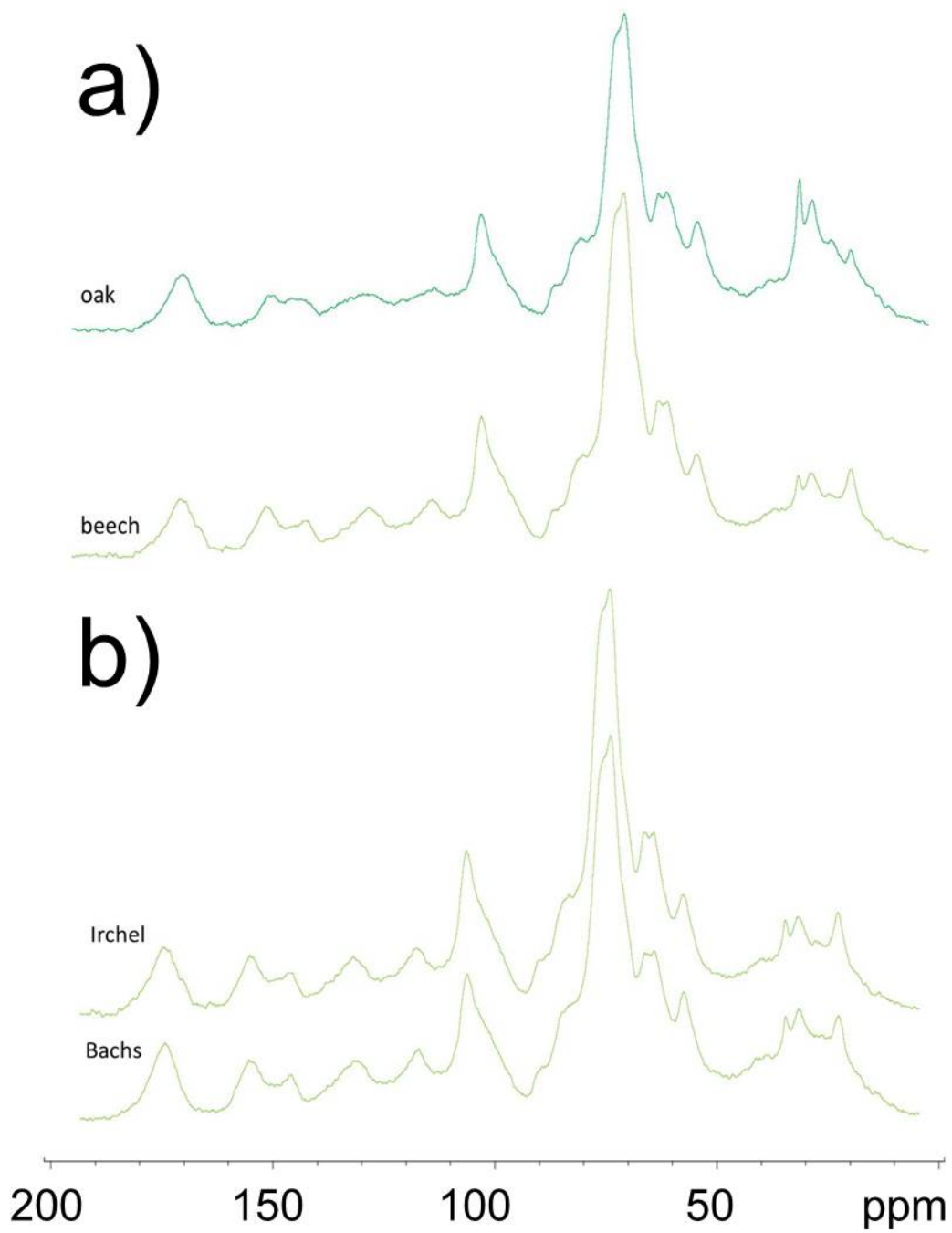
1 **Figure 5.** Cumulative cations release through 9 weeks period of leaching. Symbols indicate  
 2 means while whiskers indicate standard deviations.  
 3 Leaves comparison: diamonds indicate beech leaves while circles indicate oak leaves; open  
 4 symbols indicate Irchel while filled symbols indicate Bachs stand respectively [four  
 5 replicates].  
 6 Litter profile: diamonds indicate beech leaves, squares indicate the first mineral A horizons,  
 7 while triangles indicate individual litter horizons (the progressively paler colors indicate the  
 8 progressively more surficial horizons) [eight replicates (4 replicates • 2 sites)].  
 9 Litter removal: triangles indicate first litter horizons while squares indicate first mineral  
 10 horizons (open symbols indicate control plots) [eight replicates (4 replicates • 2 sites)].



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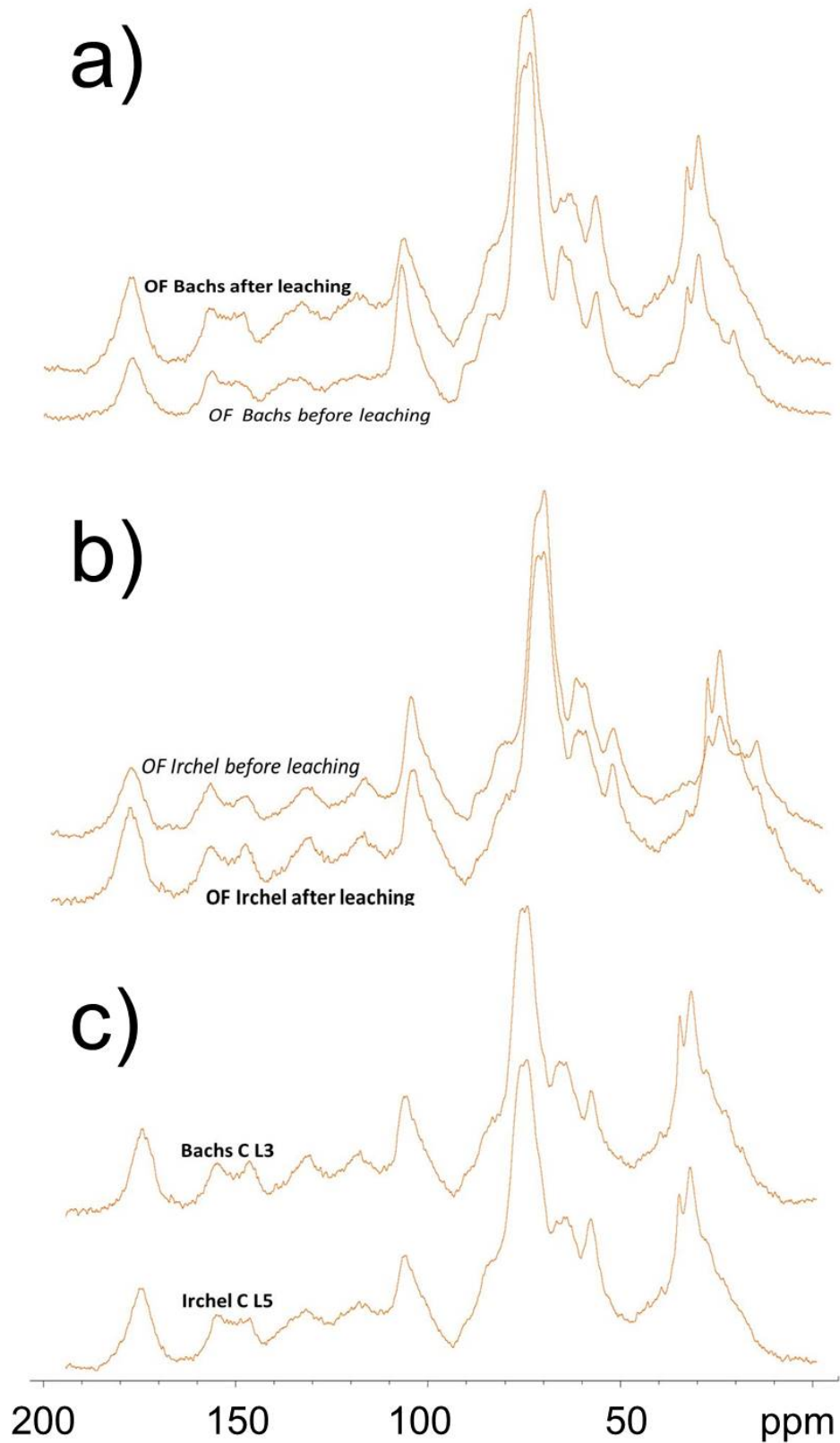
1 **Figure 6.**  $^{13}\text{C}$  CPMAS spectra. a) Bachs leaves: beech and oak b) beech leaves: Bachs  
2 and Irchel



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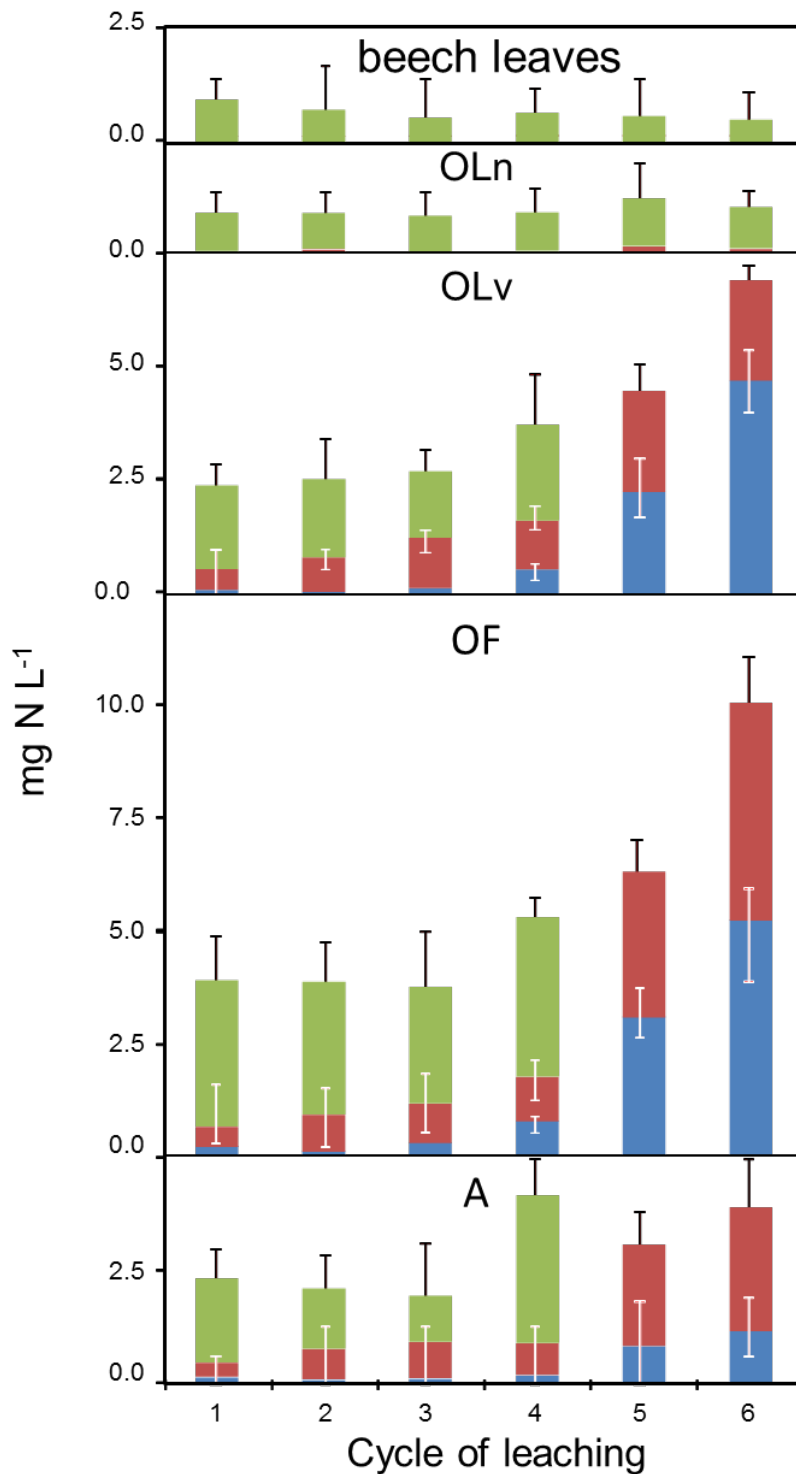
1 **Figure 7.** OF horizons  $^{13}\text{C}$  CPMAS spectra. a) Bachs: *before* and **after** leaching; b) Irchel  
2 spectra: *before* and **after** leaching; c) Bachs and Irchel.



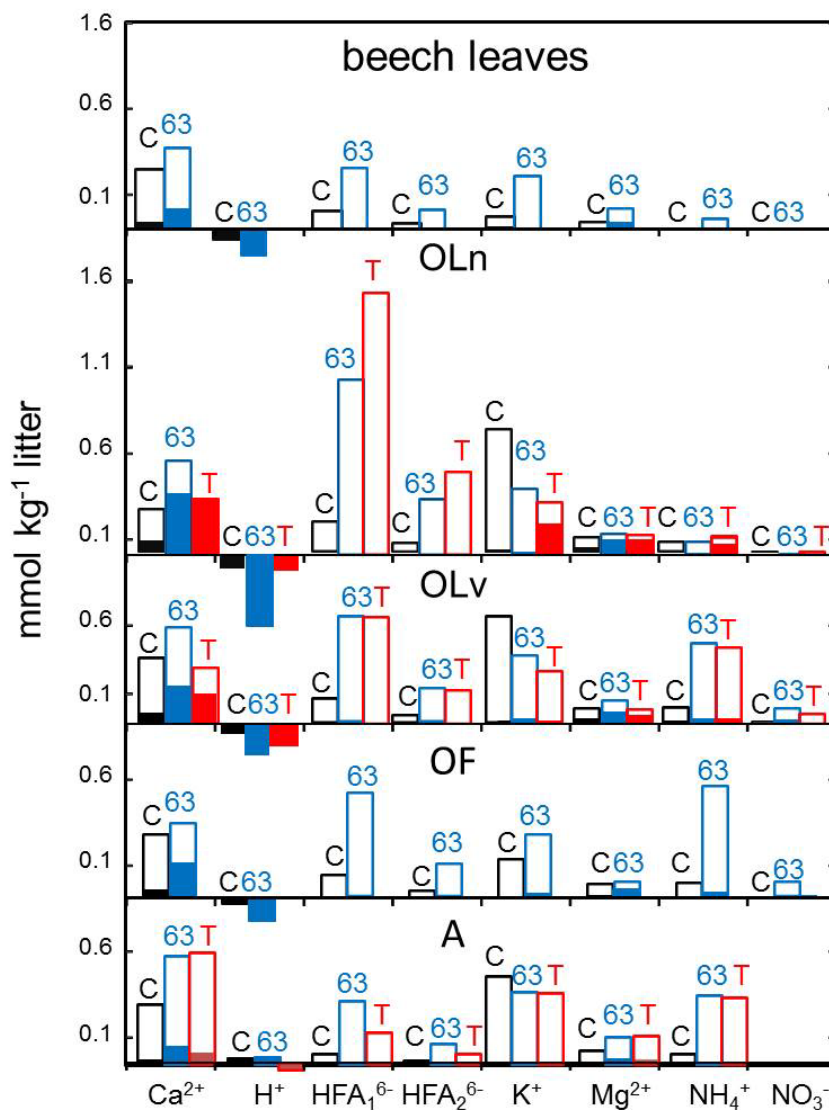
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1 **Figure 8.** Depth comparison: N concentration release through the first six cycles of  
 2 leaching. Blue histograms indicate  $\text{N-NO}_3^-$ , red  $\text{N-NH}_4^+$  while greens represent residual N,  
 3 calculated as  $\text{TN}-(\text{NO}_3^-+\text{NH}_4^+)$ . Bars indicate molar concentrations of nitrogen in leachates  
 4 and are the average of eight replicates, 4 replicate • 2 sites (whiskers indicate standard  
 5 deviation).



1 **Figure 9.** Calculated mass distributions of species from leachates. Bars indicate total  
 2 dissolved species, filled portions indicate the portions bound to DOM. Black bars indicate  
 3 control samples at time 0 of leaching (C), while blue and red bars indicate respectively  
 4 control (63) and treated with annual litter removal (T) plots after 63 days of leaching.  
 5 Nomenclature: HFA<sub>1</sub><sup>6-</sup> and HFA<sub>2</sub><sup>6-</sup> signify respectively H<sup>+</sup>-dissolved carboxylic and phenolic  
 6 fulvic acids with -6 net charge. Average molal concentrations, standard errors not shown  
 7 (always <10%).



8  
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