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REGULAR ARTICLE

Predicting nitrogen mineralization from organic amendments: beyond C/N ratio by ^{13}C-CPMAS NMR approach

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Abstract
Background and aim The processes of nitrogen (N) mineralization from organic amendments (OA) is important to provide mineral forms (NH_4^+ and NO_3^-) of N for plants. Assessment of N mineralization is usually done by using the C/N ratio of OAs but limits to the predicting capability of such indicator have been reported. Here, we propose a new definition of organic carbon (C) quality based on ^{13}C-CPMAS NMR that provide a more accurate prediction of N mineralization.
Methods A mesocosm incubation experiment was carried out to assess the effects of ten OA types on soil N mineralization in three different soils. OAs were chemically characterized by C/N ratio and in solid state spectroscopy by ^{13}C-CPMAS NMR. Nitrogen mineralization in terms of NH_4^+ and NO_3^- was monitored after 3, 10, 30, 100, and 300 days of incubation.
Results N mineralization was fast for OAs with high C quality coupled with high N content (e.g., meat powder, fish meal and alfalfa litter), while the same process was slow for amendment with low C quality even with high N content (e.g. humus, alfalfa biochar). On the contrary, a rapid but short-term N immobilization was found for OAs with high C quality but low N content (e.g., glucose). OAs with low C quality and low N content (e.g., sawdust, cellulose, wood biochar and grass litter) showed a slow, but long-lasting N immobilization. We found that C/N ratio was unable to predict the N mineralization for OAs with low C quality. Considering ^{13}C-CPMAS NMR spectral regions, the carbonyl C, and N-alkyl and methoxyl C regions had the most significant positive correlation with N mineralization, while the di-O-alkyl C and O-alkyl C were strongly associated with N immobilization.
Conclusions This study demonstrates that the biochemical quality of organic C defined by ^{13}C-CPMAS NMR is capable of predicting N dynamic pattern better than C/N ratio.

Keywords N mineralization · Organic amendment · C/N ratio · ^{13}C-CPMAS NMR · Soil quality

Introduction

Nitrogen (N) is a fundamental element for ecosystem function and, though is abundant in Earth's atmosphere, most living organisms cannot use atmospheric N_2 as a source of N. Moreover, in soil most of N exists in organic forms that, with the exceptions of plant living in cold and organic matter rich ecosystems (Näsholm

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et al. 2009), is not easily available for plants. Therefore, soil mineralization i.e. the conversion of an element from an organic form to an inorganic state as a result of microbial activity, is of great importance for plant nutrition in natural, as well as in agro-ecosystems.

N mineralization rates are strongly affected by climatic variables, with soil temperature and water availability being considered the most important factors acting at global and regional scale (Parton et al. 2007). However, at local scale with nearly uniform climate, the rate of N mineralization is mainly influenced by leaf litter or organic amendment (OA) chemical quality (Rowell et al. 2001; Masunga et al. 2016). In agro-ecosystems, crop residue chemistry varies dramatically in relation to the plant life forms (e.g., forbs, N-fixing, or graminoid) and, within species, with plant tissues (e.g., leaf, root, and woody material). The same holds for exogenous applied OA in agro-ecosystems. The chemistry of OAs widely varies, spanning from green manure and animal manure, to organic waste from the agro-industry (i.e., meat and bone meal, fish meal, paper and olive mill waste), to compost or potting substrates like peat (Bonanomi et al. 2018), until biochar, the products of pyrolysis of biomasses (Lehmann and Joseph 2015).

Since the organic carbon fraction of plant litter and OAs is chemically very heterogeneous, research tries to define their chemical composition to predict N mineralization rate (Vigil and Kissel 1991; De Neve and Hofman 1996; Berg and McClaugherty 2013). Historically, OAs chemistry was defined by the widely used ratio between C and N, the so-called C/N ratio. The C/N ratio is linked to N mineralization because it is stoichiometrically linked with the requirement of saprophytic microbes (Manzoni et al. 2008). In fact, within soil, microbes feed on organic matter requiring both organic C and N at relatively fixed stoichiometric ratio. Organic C or N can either limit microbial proliferation when C/N ratio is below, or above, the threshold value of ~30. In other words, when the C/N ratio is above ~30, microbial growth as well as organic matter decomposition and the associated N mineralization rate decrease because of N starvation. Therefore, when high C/N ratio OAs are incorporated into the soil, microbe rebalance the ratio by mineral N uptake, thus making it unavailable to plants by temporarily immobilizing it within the living microbial biomass (Aber and Melillo 1982; Hodge et al. 2000). On the other hand, when C/N ratio of AO is below ~30, organic N is readily mineralized at increasing rates inversely proportional to the value of the ratio.

C/N rapidly became the standard way to describe plant litter and OA chemistry for practical agronomic purposes as well the key variable in C-cycle models (e.g. Nicolardot et al. 2001). This occur because of its good capability to predict N mineralization in relation to other ecosystem functions like litter mass loss (Taylor et al. 1989) and its low cost and technical simplicity in being measured.

In spite of the positive features of C/N ratio, this index is not free of uncertainty and, over the last decade, several issues have emerged. Some studies reported the limitations of C/N as a predictor of leaf litter decay rate in lowland Amazonian forests (Hättenschwiler et al. 2011), as well as in temperate and Mediterranean ecosystems (Bonanomi et al. 2013). A similar finding was reported for decomposition of fine roots in a temperate forest, were the decay rate was lower for plant tissue with low C/N ratio, the opposite of what was expected (Goebel et al. 2011). Recent studies have highlighted the poor capability of C/N ratio to predict ecosystem functioning, including litter phytotoxicity (Mazzoleni et al. 2015), soil aggregate stability (Sarker et al. 2018), soil water repellency (Cesarano et al. 2016), and OA suppression of soilborne pathogens (Bonanomi et al. 2018). The main weakness of C/N ratio is due to the fact that refers to the total amount of C, lacking of any description of its biochemical composition. In fact, materials with similar C/N ratio could be composed of C type spanning from simple sugars to highly aromatic, recalcitrant compounds. Therefore, it can be hypothesized that OA with similar C/N ratios but different C quality might have different effects on soil functioning and N mineralization.

In this context, a new and accurate definition of OAs biochemical quality would be useful for an improved description of N mineralization dynamics in soil system after OAs application. To this aim, we carried out a 300 day mesocosm incubation experiment in laboratory condition to assess the effects of ten OA types on soil N mineralization rate in three soils. OAs were chemically characterized by ^{13}C cross-polarization magic angle spinning (CPMAS) nuclear magnetic resonance (NMR) in solid state, a proven method useful for relating the characteristics of soil organic matter to soil processes like aggregate stability (Piccolo et al. 2005), litter decomposition (Bonanomi et al. 2013) and plant growth (Mazzoleni et al. 2015). A considerable research effort has carried out in the last decades to find indicators of C quality that might act as reliable predictors of ecosystem functions (Cornelissen and Thompson 1997; Cornwell et al. 2008). For instance, the partitioning of organic C into different pools, broadly corresponding to structural classes of biomolecules has been attempted in many experimental and modelling works (e.g. Incerti et al. 2011). Here, we explore a more explicit representation of amendment C quality based on empirical data that associated N mineralization with ^{13}C-CPMAS NMR spectral regions.

Specific aims of the study were to:

(1) describe the magnitude and timing of N mineralization following OA incorporation in soils;
(2) explore the relationships between N mineralization rate and OA quality, as defined by ^{13}C-CPMAS NMR spectroscopy and C/N ratio; and
(3) identify the ^{13}C-CPMAS NMR spectral regions and corresponding organic C types of OA more strongly associated with N mineralization.

Materials and methods

Selection and chemical characterization of OA

Ten types of OA were selected as representing a wide range of chemical compositions (Table 1). OAs were characterized for total N and C content by flash combustion of micro samples (5 mg for each sample) in an Elemental Analyzer NA 1500 (Fison 1108 Elemental Analyzer, Thermo Fisher Scientific). Moreover, OAs were characterized by solid state ^{13}C-CPMAS NMR under the same conditions, thus allowing a quantitative comparison among NMR spectra. A spectrometer (Bruker AV-300, Bruker Instrumental Inc., Billerica, MA, USA), equipped with a magic angle spinning (MAS) probe with wide-bore of 4 mm, set up with MAS of 13,000 Hz of rotor spin, a recycle time of 1 s, and a contact time of 1 mswith acquisition time of 20 ms, and 2000 scans was used (for details see Sarker et al. 2018). Selection of spectral regions and identification of corresponding classes of organic C bonds were performed according to previous studies (Kögel-Knabner 2002; Piccolo et al. 2005; Mathers et al. 2007; Bonanomi et al. 2013). The following seven chemical shift regions representative of main C types were considered (restricted regions of relevance in brackets): 0–45 ppm = alkyl + alpha amino C; 46–60 ppm = N-alkyl C (56 ppm = methoxyl, alpha-amino); 61–90 ppm = O-alkyl C; 91–110 ppm = di-O-alkyl C (103–105 ppm = anomeric C in carbohydrate, quaternary aromatic carbons in tannins); 111–140 ppm = H- and C- substituted aromatic C (126 ppm = unsubstituted); 141–160 ppm = O-substituted aromatic C (phenolic and O-aryl C, 147–153 ppm = heterosubstituted, vanillyl+syringil lignin units); and 161–190 ppm = carbonyl C (172 ppm = carboxyl + amide, 198 ppm = ketone/aldehyde). The relative contribution of each region was determined by integration of MestreNova 6.2.0 software, Mestre-lab Research 2010, and then expressed as percentage of the total area.

Nitrogen mineralization experiment

Three soils, hereafter named S1 (Botanical garden), S2 (Capasso Farm), and S3 (Concilio Farm), showing different texture and nutrient availability, but similar organic carbon content (Table 1) were selected for the soil incubation experiment. S1 soil (40° 48′ 56.3″ N, 14° 20′ 13.8″ E, elevation 37 m a.s.l., mean annual temperature 15.8 °C and rainfall 901 mm), is a sandy soil with Andic properties (USDA Soil Taxonomy System, Soil Survey Staff 1999) collected in the greenhouse of the Agricultural Department of University of Naples Federico II. S2 soil (40°59′57.89″ N, 14°19′00.89″ E, elevation 24 m a.s.l., mean annual temperature 15.9 °C and rainfall 988 mm), is a Silandic and Vitric Andosols used for horticulture, has a loamy texture with slightly higher soil organic carbon (SOC) content. S3 soil (40° 34′ 34.67″ N, 14° 59′ 37.69″ E, elevation 41 m a.s.l., mean annual temperature 16 °C and rainfall 913 mm), is a Typic Ustifluvent with a clay-loam texture with a high

Table 1 Physical and chemical properties (mean values of three replicates) of S1, S2 and S3 soil types at the beginning of the experimental activity

Parameter	S1	S2	S3
Sand, %	60.38	45.60	23.48
Silt, %	21.22	46.42	40.14
Clay, %	18.40	7.98	36.38
Bulk density, g cm^{-3}	1.37	1.19	1.46
Electrical conductivity, dS m^{-1}	0.14	0.61	0.29
pH	6.25	7.72	8.07
Organic carbon, g kg^{-1}	9.68	13.14	10.20
Total nitrogen, g kg^{-1}	4.45	1.91	3.10
C/N ratio	2.17	7.38	3.27

pH and a medium soil organic carbon content (USDA Soil Taxonomy System, Soil Survey Staff 1999). In each farm, five soil sub-samples were collected following a W scheme that was thereafter pooled in order to have a single sample for each study site. Soil sample (~20 kg from each farm) were packed in polyethylene bags, transferred to laboratory, sieved at 2 mm and air-dried at 30 °C.

The mineralization experiment was carried out in mesocosms. Plastic jars (diameter 9 cm, total volume of 500 cm^3) were filled with 100 g of dry soil to which were incorporated 2 g (2% *w*/w) of each dry OAs type and thoroughly mixed. Experimental values of OAs addition fall within the range of amendment applied in several agricultural systems. All OAs types were previously dried and ground to 2 mm. Mesocosms were kept in a growth chamber under controlled temperature (18 ± 2 °C night and 24 ± 2 °C day) and moisture content was monitored regularly and watered every seven days to field capacity with distilled water. While watering to field capacity is debatable when applied to standardize experimental conditions for different soils, it was considered suitable in our experiment since it was applied to soil types with similar retention curves, as shown elsewhere (Bonanomi et al. 2017).

Both amended soil treatments and not amended controls were incubated for 3, 10, 30, 100, and 300 days. The full experimental design included three soil types, ten organic amendments and one control, triplicated for each of 5 incubation times, for a total of 495 experimental units. At each sampling date, 99 experimental units were destructively sampled, the soil was collect, air dried and submitted to chemical analysis.

Soil chemical analysis

Incubated soil samples were prepared for NO_3^- and NH_4^+ content determination by mixing 1 g of dry pulverized soil with 1 ml of distilled water in a 2 ml Eppendorf tube. This mixture was shaken for 20 min and subsequently centrifuged for 5 min at 13,000 rpm. The samples were analyzed with a DR 3900 Spectrophotometer (Hach, Loveland, CO, USA) by using the manufacturer kits LCK 340 (assay range 5–35 mg/l, ISO 7890-1-2-1986) for NO_3^- and LCK 303 (assay range 2–47 mg/l, ISO 7150-1) for NH_4^+. Measurements were taken for all 3 soils, all sampling dates (at 3, 10, 30, 100 and 300 days from OA application) and triplicates were performed for each assay activity.

The pH and Electrical conductivity (EC) values of soil samples were determined by standard methods (Sparks 1996). Dry pulverized soil mixed with distilled water at 1:2.5 ratios and then set on electronic shaker for one hour. The pH and EC of water-soil mixture was determined using a BASIC 30, CRISON pH-meter and BASIC 30, CRISON conductometer, respectively.

Statistical analysis

In order to explore the chemical variability of the OAs, a data matrix of signals recorded in their ^{13}C-CPMAS NMR spectra was submitted to Cluster Analysis using complete linkage as linking rule and Pearson's correlation coefficient as a similarity measure. Data from the nitrogen mineralization experiment were submitted to Generalized Linear Modelling (GLM), considering main and interactive effects of soil type (S, three levels), organic amendment (OA, ten levels) and incubation time (treated as a continuous covariate) on soil NH_4^+, NO_3^-, pH and EC changes.

To address the relationships between OA and mineral N forms (NH_4^+ and NO_3^-) released at different incubation dates, different approaches were considered. First, simple linear correlation analysis was separately tested between NH_4^+ and NO_3^- at 3, 10, 30, 100 and 300 days in each soil and OA chemical compositions, including both physico-chemical features (i.e. C, N, C/N, pH and EC) and regions of the ^{13}C-CPMAS NMR spectra selected from reference literature (Kögel-Knabner 2002; Mathers et al. 2007).

In a more detailed analysis, correlation analysis was extensively carried out for N forms (NH_4^+ and NO_3^-) in the tested soil amended with 10 amendment types and ^{13}C NMR signals recorded for the same organic materials at each resonance signal ($n = 190$). This analysis provides a fine-resolution profile of the effect in C types on NH_4^+ and NO_3^- mineralization and elucidates any significant correlation of NH_4^+ and NO_3^- release with restricted ^{13}C-CPMAS NMR spectral signals. The correlations were tested for statistical significance controlling for multiple comparisons, according to Bonferroni's correction at $p < 0.01$.

Principal Component Analysis (PCA) was carried out in order to provide a synthetic representation of the relationships between OA chemistry and N mineralization at different incubation dates. PCA was carried out on a data matrix of the spectral regions in the OA, selected from reference literature (as described above) and by physical-chemical features. In the PCA, the NH_4^+, NO_3^- content at 3, 10, 30, 100 and 300 days were included as supplementary variables (i.e. plotted in the multivariate space but not used to calculate the principal components), following the approach suggested by Legendre and Legendre (1998). GLM was done using IBM SPSS Statistics 24, whereas Statistica 7 was used for all other analyses.

Results

OA initial chemistry

The OA types showed a wide range of chemical quality in terms of element content, and different C-forms as revealed by ^{13}C-CPMAS NMR (Table 2 and Fig. 1a). Considering C bond types, alkyl-C and methoxyl and N-alkyl C fractions showed highest peaks in fish meal and meat powder, followed by humus and alfalfa litter. On the other hand, these regions were less pronounced in grass litter, sawdust, and both of the biochars, being substantially absent in cellulose and glucose. The O-alkyl-C and di-O-alkyl-C, largely associated with polysaccharides and sugars were abundant in glucose, cellulose, sawdust, grass litter, followed by other amendments, while in humus and the two biochars these molecular types were not detected. The H- and C-substituted aromatic C was the most abundant in wood biochar, alfalfa biochar, followed by humus, but showed a much lower content in all other materials. The O-substituted aromatic C showed minor or no peaks in most OA types, whereas carbonyl C was very abundant in meat powder, alfalfa litter and, to a lesser extent, in fish meal, alfalfa biochar and humus.

The dendrogram derived from cluster analysis allowed a comparison among the OA types, either in terms of spectral signals and corresponding C bond types (Fig. 1b). Meat powder, glucose, and humus showed dissimilarity from all other OAs, as well as each other. Similarities were observed between alfalfa litter and fish meal, and between alfalfa biochar and wood biochar, as well as between grass litter, sawdust, and cellulose.

Effect of OAs on N mineralization, soil pH and EC

In the N mineralization experiment, all treatment factors (i.e. type of soil and amendment, incubation time) significantly affected soil mineral N forms (NH_4^+ and NO_3^-), by either main or interactive effects (Supplementary Table S1). In general, the application of OAs affects soil mineral N with timing and magnitude that were highly variable among the tested materials (Figs. 2 and 3). Among OAs types, fish meal and meat powder showed high NH_4^+ concentration in all soil types, followed by alfalfa litter (Fig. 2). Specifically, high NH_4^+ soil concentration was observed with fish meal, meat powder and alfalfa litter after 3, 10, and 30 days of incubation. Thereafter, the soil NH_4^+ concentration progressively decreased. In contrast, OAs like cellulose, grass litter, glucose, saw dust and wood biochar showed no changes in NH_4^+ compared to the control in all soil types (Fig. 2). Humus extract showed a different pattern, with high NH_4^+ concentration after 30 and 100 days of incubation. Alfalfa biochar showed a limited increase of NH_4^+ concentration, compared to the control, after 100 and 300 days of incubation in S3 soil (Fig. 2).

Considering NO3-, fish meal, meat powder, and alfalfa litter showed a significant NO3 release in all soil types. Specifically, the soil NO3- concentration was higher, compared to the control, following the application of with these OAs after 30 and 100 days of incubation, with a significant but smaller difference after 300 days (Fig. 3). Humus extract showed a continuous increase of soil NO_3^- concentration over time, reaching the highest value at 300 days in all soil types. Cellulose, grass litter, glucose, saw dust, and wood biochar immobilized NO_3^- during the incubation period. Glucose application reduced NO_3^- concentration after 3 and 10 days of incubation, showing values similar or slightly lower than the control thereafter (Fig. 3). Cellulose, grass litter, and sawdust application showed the lowest soil NO_3^- concentration after 100 days of incubation. However, in case of sawdust application, soil NO_3^- concentration remained very low even after 300 days of incubation. Finally, alfalfa and wood biochar showed a short- and medium-term N immobilization. Specifically, alfalfa biochar decreased soil NO_3^- concentration after 3, 10 and 30 days while, in case of wood biochar, the concentration decreased after 100 and 300 days compared to the control.

OAs significantly affected soil pH (Supplementary Fig. S1). Meat powder increased pH, compared to

Table 2 Initial C, N content and C/N ratio, as well as NMR spectral regions of 10 different organic materials used in the N mineralization experiment. The relative contribution of each NMR region was expressed as percentage of the total area

Parameters	Alfalfa litter	*Alfalfa biochar	*Wood biochar	Cellulose	Fish meal	Glucose	Grass litter	Humus	Meat powder	Saw dust
Elemental										
C (%)	38.29	52.25	74.57	50.10	50.19	43.00	45.34	35.90	43.88	49.88
N (%)	3.93	4.43	0.50	0.10	6.06	0.00	1.19	2.40	8.26	0.11
C/N ratio	9.73	11.79	149.14	501.00	8.28	∞	38.10	14.96	5.31	453.45
^{13}C-CPMAS NMR										
Carbonyl C	11	8	7	1	9	4	4	8	19	2
O-subst. Aromatic C	2	14	4	1	2	4	3	8	2	3
H-C-subst.aromatic C	6	46	61	2	5	6	9	33	4	7
di-*O*-alkyl C	8	9	7	16	6	15	15	5	1	14
O-alkyl C	38	10	5	74	31	61	55	4	8	56
Methoxyl C	10	3	4	3	16	2	5	6	20	8
Alkyl C	22	7	9	1	27	11	6	33	41	6
^{13}C NMR indices										
Alkyl C /O-alkyl C	0.59	0.71	1.67	0.01	0.88	0.18	0.12	7.04	4.65	0.11
Carbonyl C /Methoxyl C	1.10	2.37	1.86	0.06	0.57	1.81	0.88	1.35	0.93	0.36

*Alfalfa biochar (from alfalfa leaves and stalks) and wood biochar (from chopped *Fagus sylvatica* wood) were produced by pyrolysis at 550 °C for 5 h in a muffle furnace

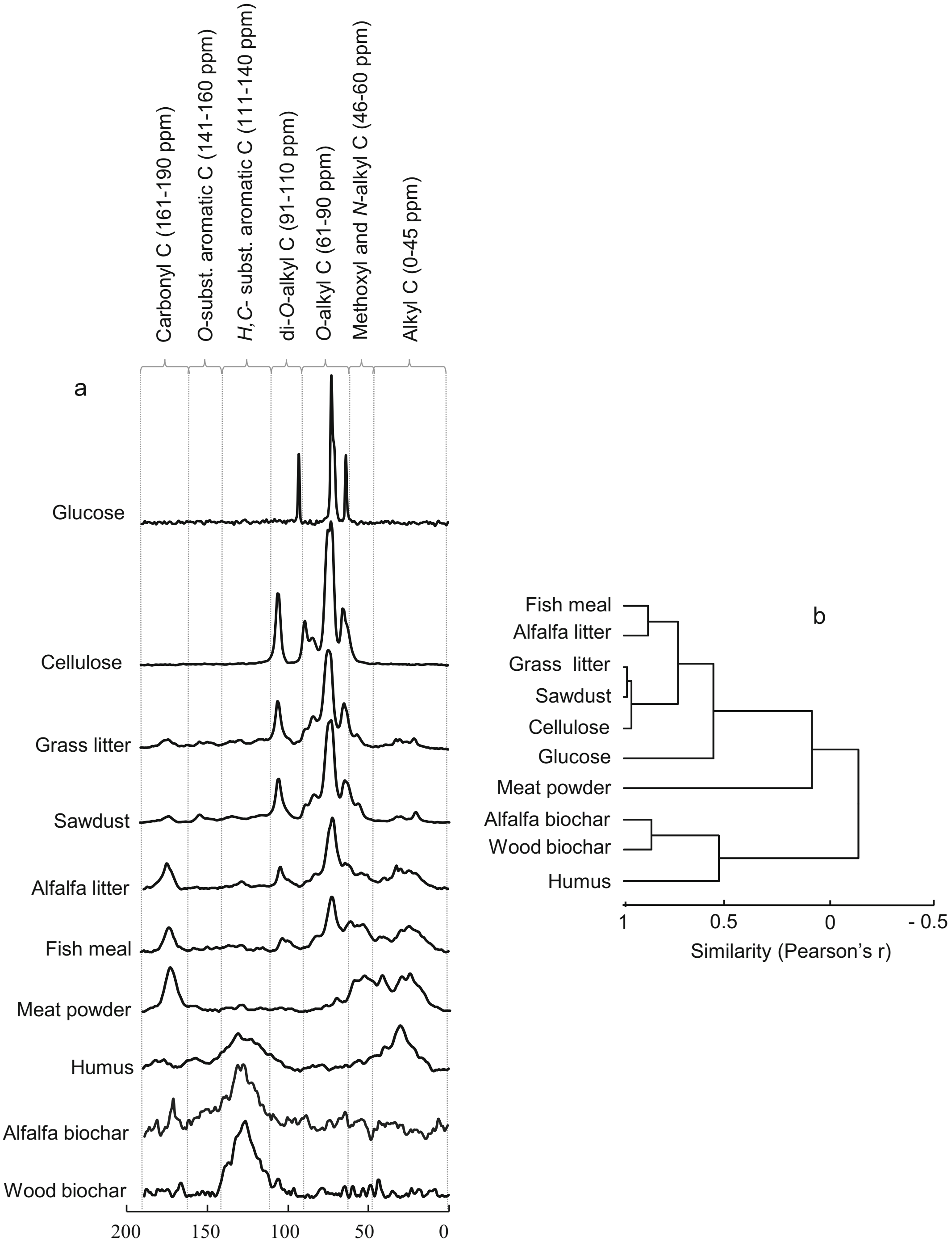

Fig. 1 Chemical quality of organic materials: (**a**) ^{13}C-CPMAS NMR spectra, reference spectral regions and corresponding C types are reported on top of the panels, with chemical shift ranges indicated in brackets and by vertical dotted lines. **b** Dendrogram of organic materials obtained by hierarchical cluster analysis (HCA) applied to ^{13}C-CPMAS NMR data

control, in the early decomposition phases but acidified the soil in later stages. A similar pattern was observed with glucose. Fish meal reduced pH only after 300 days of incubation. Humus and alfalfa biochar increased pH during the whole incubation period while other OA had little effect on pH.

Soil EC was largely affected by OA application, incubation time, and soil type (Supplementary Fig. S2). EC was increased by the application of meat powder, fish meal, alfalfa litter, alfalfa biochar, and humus. Glucose, on the other hand, increased soil EC only after 300 days of incubation. Soil amendment with sawdust, cellulose, and grass litter generally decreased EC, with wood biochar having little effect on this parameter.

Relationship between OA chemistry and N mineralization

Soil mineral N forms turned out to be correlated with several OA parameters, but the sign and statistical significance was strongly dependent by the incubation time (Figs. 4a and 5a). Heat maps showed that initial N in OA had significant positive correlation with soil NH_4^+ and NO_3^-, while C and C/N ratio showed a general pattern of not significant correlation, with some exceptions in later incubation stages. The soil pH showed a general pattern of not significant correlation with soil NH_4^+, while a significant negative correlation with NO_3^- was found only in S2 soil and in the later incubation time. EC had significant positive correlation with NH_4^+ only in S1 soil at 100 days, with few positive correlations in the case of NO_3^-.

When OA chemical quality was molecularly defined by ^{13}C-CPMAS NMR, we observed a remarkable pattern of correlation with soil mineral N forms with specific C bond types (Figs. 4a and 5a). We observed robust trends of significant positive correlation for alkyl C, methoxyl C and carbonyl C with soil NH_4^+ and NO_3^-. Considering soil NH_4^+, these three regions had significant positive correlation up-to 100 days in all soils, as well as their average, while in S2 soil carbonyl C showed a significant correlation throughout the incubation period. In the case of NO_3^-, significant positive correlations were observed from the mid to later stages, starting at 10 days in S1 and S2 soil, and throughout the incubation period in S3. On the contrary, strong negative correlations were recorded for O-alkyl-C and especially di-O-alkyl-C with soil mineral N forms. O-substituted aromatic C and H, C-substituted aromatic C showed a general pattern of not significant correlation with soil NH_4^+, although S3 soil showed a significant correlation at 300 days. These two aromatic C regions showed a general pattern of not significant correlation with soil NO_3^-, although O-substituted aromatic C had significant negative correlation at 3 days for all soil types. The carbonyl C/methoxyl and alkyl C/O-alkyl-C ratios had few significant correlations with soil NH_4^+ and NO_3^- during the incubation time (Figs. 4a and 5a).

The PCA results provide a synthetic picture of the chemistry-dependent effects on soil mineral N content at different incubation dates (Figs. 4b and 5b). Overall, it was confirmatory of the above-described correlations between OAs chemistry and mineral N forms. Moreover, PCA clearly showed that the association between OAs chemistry and soil NH_4^+ and NO_3^- shifted during incubation time, both for elemental chemical features and for ^{13}C NMR derived parameters.

The detailed correlation profiles of soil mineral N forms with the intensity of single NMR signals provided further insight into the relationships between the molecular composition of OAs and soil NH_4^+ and NO_3^- content (Fig. 6a, b). Different restricted ^{13}C-NMR spectral sections were strongly correlated with soil N mineral forms. The correlation profiles of NH_4^+ were similar in the time interval 3–100 days but largely deviated after 300 days of incubation (Fig. 6a). Briefly, up-to 100 days, soil NH_4^+ showed a significant positive correlation with restricted intervals within the alkyl C (15–25 and 40–46 ppm), the whole methoxyl C, and one restricted interval within the carbonyl C (165–175 ppm) region. On the other hand, significant negative correlations were observed with two restricted intervals within the di-O-alkyl-C (105–110 ppm). The correlation profiles of soil NO_3^- showed similar pattern for 10, 30, 100 and 300 days, while exception was observed after 3 days of incubation (Fig. 6b). Significant positive correlations were observed with restricted intervals within the alkyl C (10–20 ppm), methoxyl C (50–57 ppm), and carbonyl C (172–180 ppm). Moreover, significant negative correlations were recorded for restricted intervals within the di- O-alkyl C (105–110 ppm).

Discussion

Nitrogen mineralization from OA and the weakness of the C/N ratio

Mineral N release following the application of organic amendments to soils has been extensively investigated (Trinsoutrot et al. 2000; Cabrera et al. 2005; Cayuela et al. 2009; Masunga et al. 2016). Our study confirmed that the dynamics and magnitude of the N release or

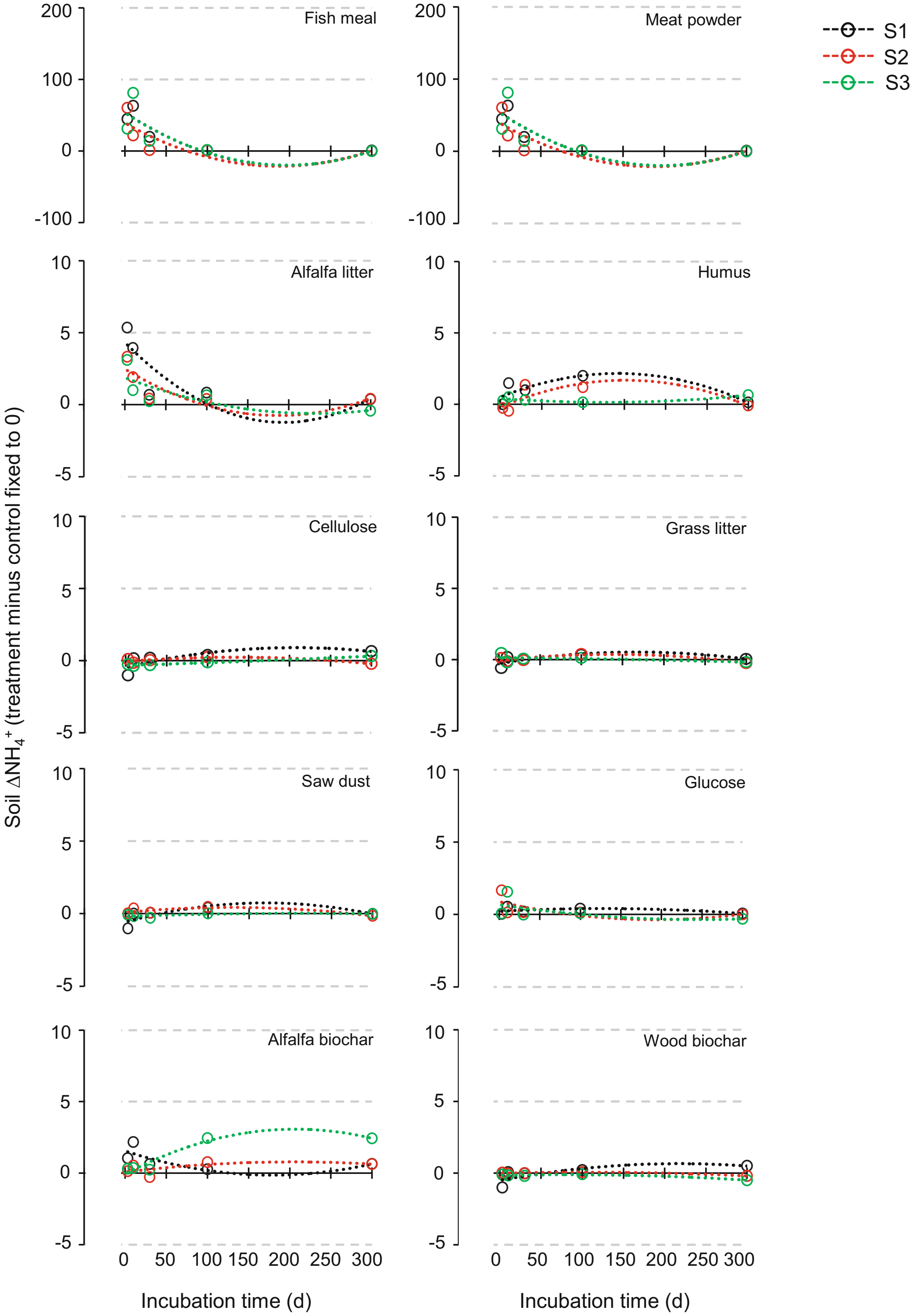

Fig. 2 Soil ΔNH_4^+ (treatment minus control fixed to 0) in three soils amended with 10 organic materials (alfalfa litter, alfalfa biochar, cellulose, fish meal, glucose, grass litter, humus, meat powder, sawdust and wood biochar) during 300 days of incubation period. Note different y-axis scales

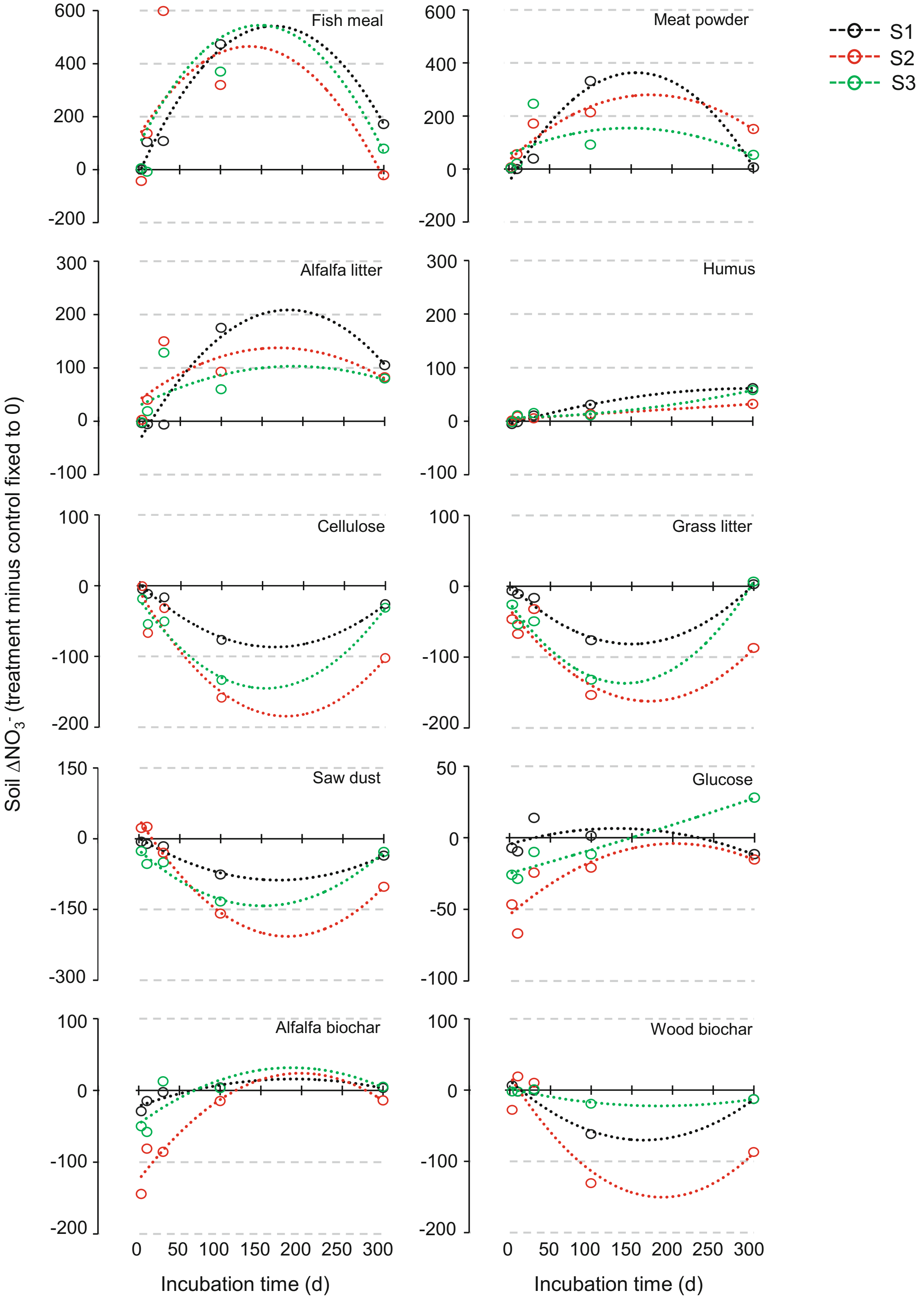

Fig. 3 Soil ΔNO_3^- (treatment minus control fixed to 0) in three soils amended with 10 organic materials (alfalfa litter, alfalfa biochar, cellulose, fish meal, glucose, grass litter, humus, meat powder, sawdust and wood biochar) during 300 days of incubation period. Note different y-axis scales

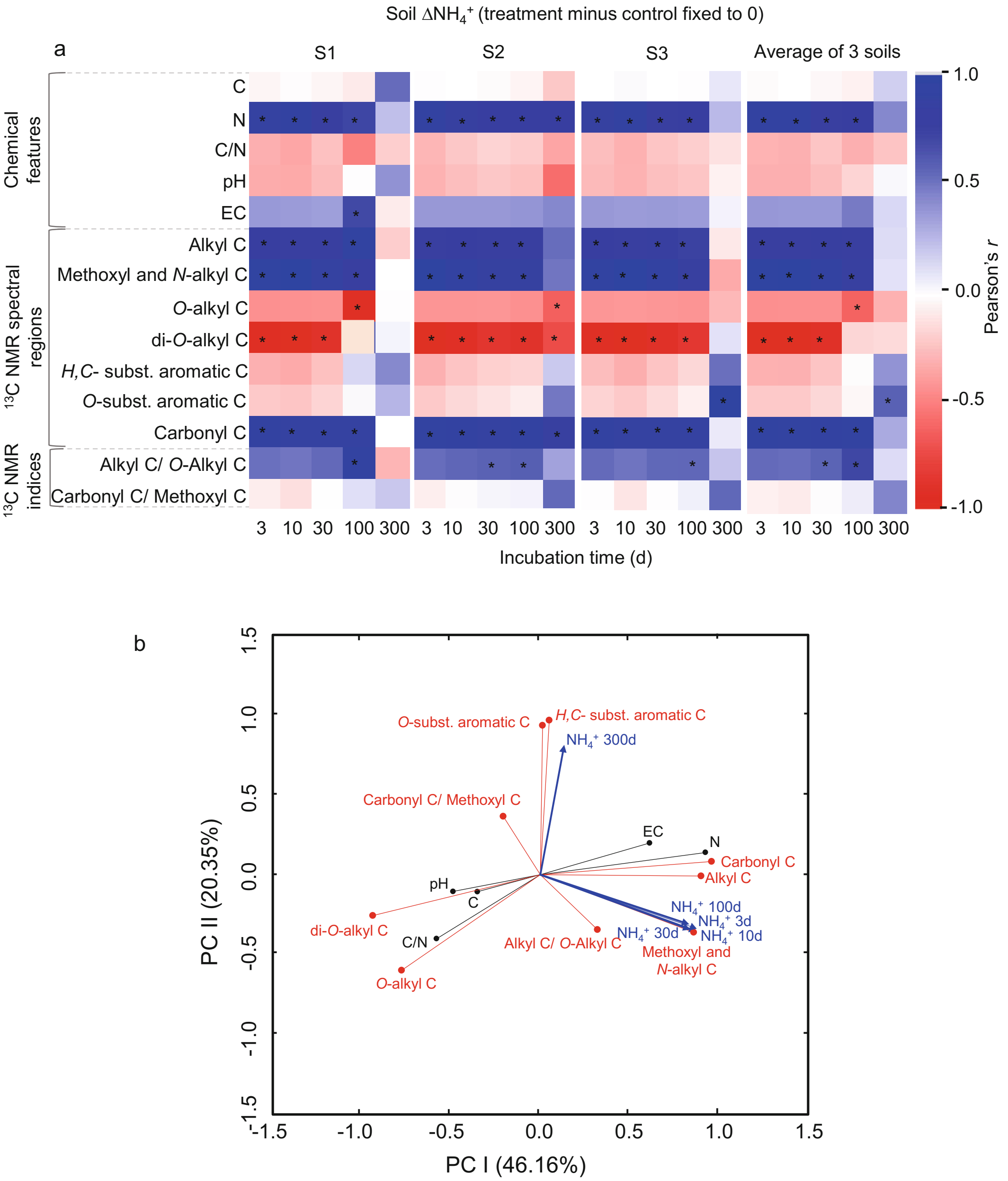

Fig. 4 **a** Heat-plot of correlation (Pearson's *r*) between soil ΔNH_4^+ observed in the 3 soils and their average at 3, 10, 30, 100, and 300 days, and OA chemical features (C, N, C/N, pH, and EC), main organic C types assessed by ^{13}C-CPMAS NMR. Asterisks indicate statistical significance for *r* ($p < 0.01$, after controlling for multiple comparisons according to the Bonferroni's correction). b) Principal component analysis (PCA) of OA ^{13}C-CPMAS NMR regions (red vectors) and elemental chemical traits (black vectors). Soil ΔNH_4^+ observed in soils at 3, 10, 30, 100 and 300 days is plotted as supplementary variable (blue vectors) following Legendre and Legendre (1998)

immobilization is highly variable between OAs, with a strong incubation time effect depending on their chemical composition. We found that OAs can be categorized in four main groups based on N mineralization timing. The first group (fish meal, meat powder, and alfalfa litter) rapidly mineralized a large amount of N, producing a notable flush of NH_4^+ shortly after their application. The second group included just humus showed a slow N release. On the other hand, six OAs immobilized N: glucose and alfalfa biochar

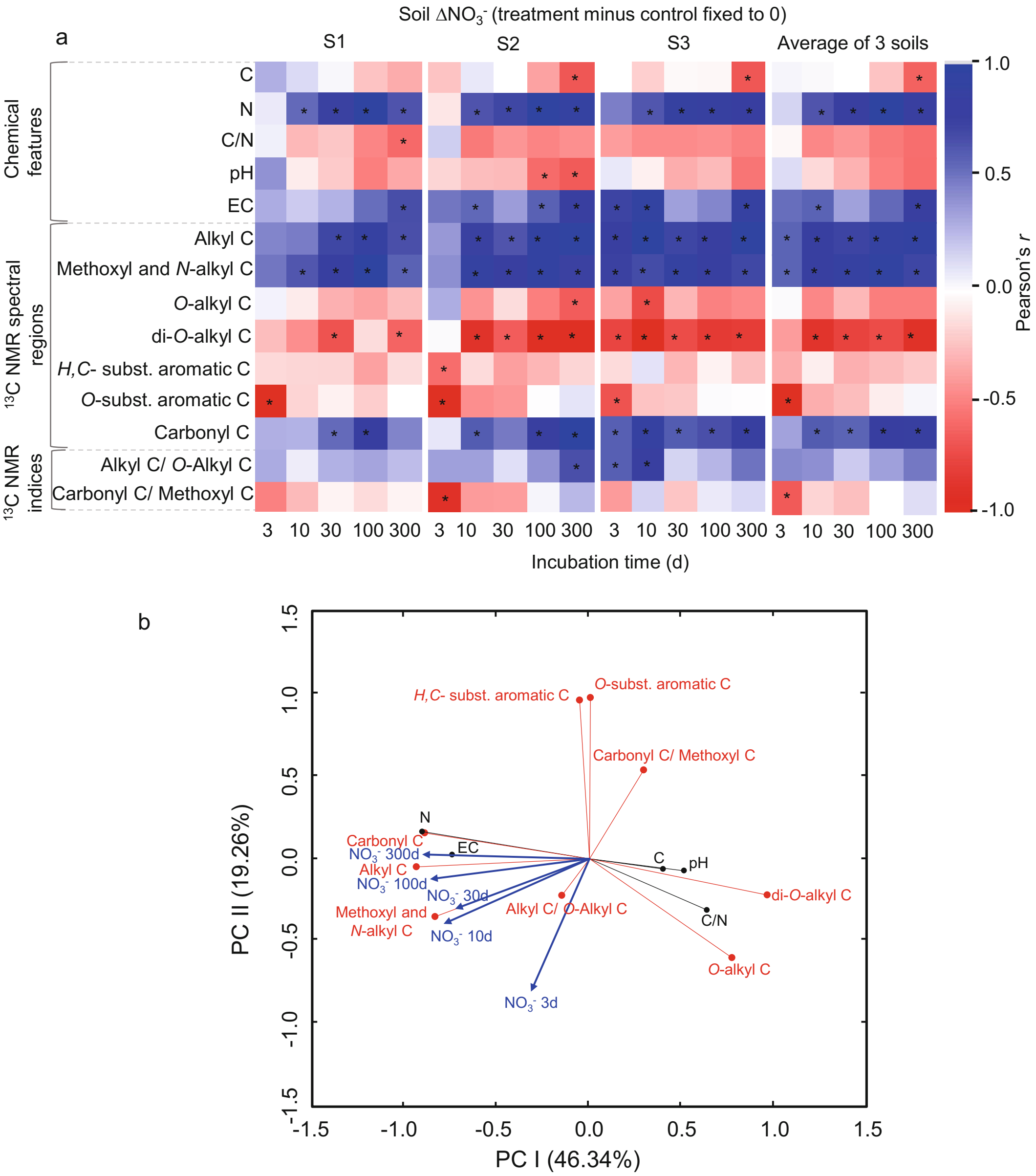

Fig. 5 **a** Heat-plot of correlation (Pearson's *r*) between soil ΔNO_3^- observed in the 3 soils and their average at 3, 10, 30, 100, and 300 days, and OA chemical features (C, N, C/N, pH, and EC), main organic C types assessed by ^{13}C-CPMAS NMR. Asterisks indicate statistical significance for *r* ($p < 0.01$, after controlling for multiple comparisons according to the Bonferroni's correction). **b** Principal component analysis (PCA) of OA ^{13}C-CPMAS NMR regions (red vectors) and elemental chemical traits (black vectors). Soil ΔNO_3^- observed in soils at 3, 10, 30, 100 and 300 days is plotted as supplementary variable (blue vectors) following Legendre and Legendre (1998)

showed a rapid short-term immobilization, whereas cellulose, maize litter, sawdust and wood biochar required more time to immobilize N with the effect lasting for longer periods.

An almost immediate release of extractable NH_4^+ and a subsequent rapid conversion into NO_3^- mineralization from OAs derived from animal tissue as reported in this study is in agreement with several previous reports (Cayuela et al. 2009; Mondini et al. 2008; Tenuta and Lazarovits 2004). A similar pattern of N release was also reported for leguminous plant residues (Fox et al. 1990; Johnson et al. 2007; Nakhone and Tabatabai 2008). The behavior of the OA with low C/N ratio is largely in agreement with the standard C/N

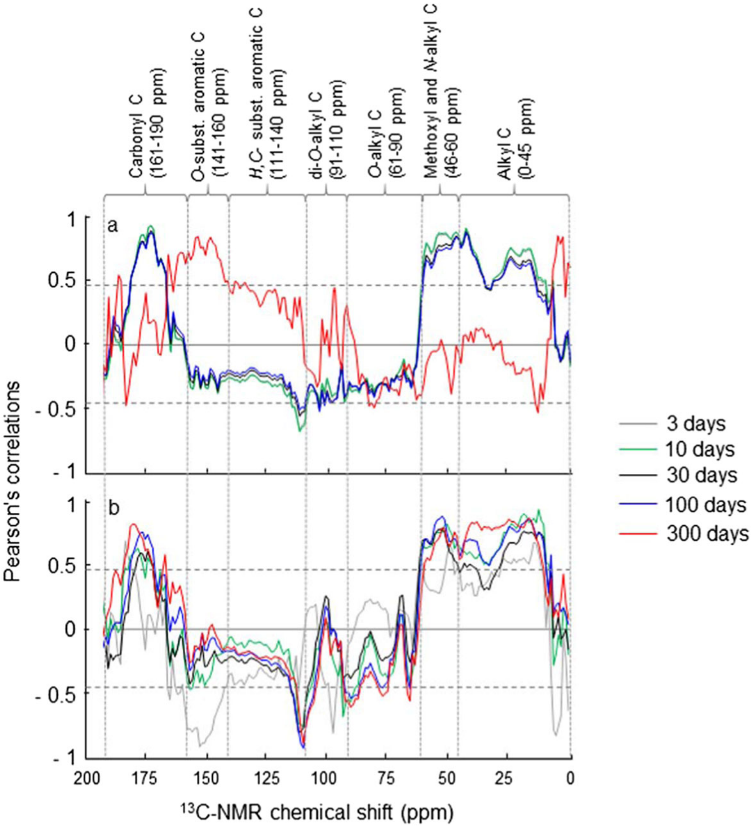

Fig. 6 Profiles of correlation (Pearson's) between ^{13}C-CPMAS NMR signals and ΔNH_4^+ (**a**) and ΔNO_3^- (**b**), in soil (average of the three soils) at 3, 10, 30, 100 and 300 incubation days. Dashed grey horizontal lines in each panel indicate threshold values of statistical significance for r ($p < 0.01$, after controlling for multiple comparisons according to the Bonferroni's correction). Organic C types assessed by ^{13}C NMR and corresponding to different spectral regions are indicated on the top of the panel and with gray dotted vertical lines

predictions. In contrast, humus mineralizes N at a much slower rate, with significant increases of soil NO_3^- only after 100 days of incubation. Slow N mineralization from humus or other biologically stabilized OAs having low C/N ratios has been previously reported (Amlinger et al. 2003; Prescott 2005) without satisfactory explanations for such slow rates. In our case, humus had quite low C/N ratio, a value like that of alfalfa litter, meat powder and fish meal, for which large N mineralization would be expected. The key to understanding such differences is provided by the assessment of carbon compound quality. ^{13}C-CPMAS NMR analysis showed that humus has a substantially different C quality than fish meal, meat powder, and alfalfa litter. Humus showed a high aromatic fraction coupled with relatively low content of O-alkyl C and di-O-alkyl C fractions associated with sugar and polysaccharides. In addition, humus C is not readily accessible to microbes, in contrast to fish meal and meat powder despite their similar low C/N ratio. The slow N mineralization from humus could be explained with low C accessibility to microbe that likely limit decomposition as well the rate of N mineralization.

On the other hand, we found that glucose, cellulose, grass litter and saw dust immobilized soil N, with an effect strongly dependent on incubation time. These results, well known in literature (Hart et al. 1994; Szili-Kovács et al. 2007; Tilston et al. 2009; Hodge et al. 2000), at first sight seem consistent with the usual C/N ratio prediction because all these materials had a $C/N > 38$. However, glucose induces rapid, short-term immobilization, while sawdust induces a slow but persistent N immobilization, with grass litter and cellulose showing an intermediate behavior. Although C/N varied widely among the four materials, these differences can hardly explain the observed differences in N mineralization. In this regard, C quality described by ^{13}C-CPMAS NMR helps to explain the observed differences in N immobilization patterns. Glucose is, obviously, the most accessible C source to microbes but lacks of N. Although microbial activity was not measured in this study, we suspect that the very rapid (3–10 days) N immobilization could result from the uptake of growing microbes that are forced to use the available soil mineral N to metabolize the exogenously applied organic C. However, this N immobilization was short-term and transient, since the mineral N content went back to the level of the control after ~100 days. After the rapid glucose consumption, the microbial turnover probably releases the immobilized N back to the soil. Further studies are needed to confirm this explanation by measuring C and N of the microbial biomass at different incubation dates.

Next, cellulose and sawdust had a similar, very high C/N ratio but rather different N immobilization patterns. Cellulose induced N immobilization after just 3–10 days while with sawdust this was evident only after 100 days of incubation. Despite the similar C/N ratio, C accessibility is much higher for cellulose than for sawdust, thus explaining the faster N immobilization dynamics. Sawdust is rich in lignin, as highlighted by the relatively high aromatic fraction observed in the ^{13}C-CPMAS NMR spectra but also in cellulose as indicated by the O-alkyl C and di-O-alkyl C regions. In fact, the slow N immobilization by sawdust can be partially explained by the large proportion of plant cellulose trapped within lignin, so that its decomposition cannot proceed independently of lignin degradation (Adair et al. 2008).

The two studied biochars, had contrasting C/N ratios (around 12 and 150 for those derived from alfalfa stalk and chopped wood, respectively) and again showed N mineralization patterns not consistent with C/N ratio theory. In the case of alfalfa biochar, substantial N mineralization was expected, which sharply contrasted with the observed short-term N immobilization. For wood biochar, a significant N immobilization would be expected, but this pattern was confirmed only after 100 days of incubation. The lack of short-term N immobilization for wood biochar could be associated with the recalcitrant nature of pyrogenic C (Lehmann and Joseph 2015). In fact, the 13C-CPMAS NMR spectra revealed that most organic C in biochars is aromatic, thus hardly accessible in the short-term to soil microbes. In literature, a large variability is reported on the effect of biochar on N dynamics, ranging from a net N mineralization to immobilization (Tammeorg et al. 2012; José and Knicker 2011; Ameloot et al. 2015). The variability of biochar chemistry, related to difference in initial feedstock quality as well as to pyrolysis conditions (i.e. temperature, oxygen availability, and duration of the thermal treatment), indeed contributes to the variable N mineralization pattern observed in literature.

Towards an improved definition of OA chemistry to explain N mineralization

According to our findings, the C/N ratio appears to be a suitable index to predict N mineralization only for OA composed of labile, easily accessible organic C. As the proportion of organic C resistant to microbial attack into OA increases, the capability of C/N index to describe N mineralization pattern drops down. Specifically, Pearson correlation coefficients between OAs and soil nitrate concentrations for the three soils were − 0.07, −0.35, −0.31, −0.38, and − 0.41 after 3, 10, 30, 100, and 300 days of incubation, respectively. Overall, the low and variable correlation values highlights the limitations of the C/N ratio and, at the same time, the importance of considering the concept of C quality for a better understanding of N mineralization. To advance in this direction, we identify, by thorough correlation and multivariate analyses, the ^{13}C-CPMAS NMR regions significantly associated with either N mineralization or immobilization.

Initially, the ^{13}C-CPMAS NMR spectral regions from literature (Spaccini et al. 2000; Almendros et al. 1994; Kögel-Knabner 2002), showed promising statistically significant relationships with N mineralization. Mineral N release, especially NH_4^+, showed very strong and positive association with the abundance of fractions mainly composed of organic acids and the amide carbon (NMR spectral regions corresponding to carbonyl C). This result indicates that the high proportion of amide carbon in some fresh OA types (e.g., meat powder, fish meal, and alfalfa litter) may lead to rapid initial N release. Besides, for these materials, the rapid N release decreased after an initial peak suggesting that these compounds might be short–lived and rapidly subjected to chemical or microbial breakdown. In this work, we also found consistent positive correlations between N release and ^{13}C-CPMAS NMR regions related to proteins and peptides (methoxyl and N-alkyl C region) and the aliphatic fraction of the NMR spectra (alkyl C). These results can be related to the degradation of proteins and lipids which are major components of meat powder, fish meal and N–fixing leaf litter as well (Fig. 1). These consistent correlations of N release and ^{13}C-CPMAS- NMR regions, supported the idea that C types could be useful for an improved OAs index predictive of N mineralization.

N mineralization was negatively correlated with simple sugars, other carbohydrates, and cellulose (^{13}C-

NMR spectral regions corresponding to di-O-alkyl C and O-alkyl C), as well as with the aromatic C fractions (O-substituted aromatic C and H, C-substituted aromatic C). We hypothesize that the negative impact of labile and aromatic C fractions on N mineralization are, however, different mechanisms. As sugars and carbohydrates are easily accessible to microbial consumption, the fast-growing microbial biomass will consume the available soil nutrients to meet their nutritional requirement, thus causing a rapid N immobilization. This effect will be more intense as C accessibility increase and as the N content of the OA decreases. However, the negative correlation between aromatic C types and N mineralization can be related to the limited accessibility of this organic fraction to microbial decomposition. In fact, this C is resistant to most forms of microbial attack (Kögel-Knabner 2002) and the high amount of recalcitrant aromatic C has been suggested to reduce the rate of N mineralization (Vigil and Kissel 1995). For lignin rich but N poor OAs, such as sawdust, wood debris, large roots, some leaf litter, we hypothesize that the prevalent aromatic C fraction slows down microbial colonization also affecting the N uptake from the surrounding soil and delaying the occurrence of N immobilization. In contrast, for N rich OAs with a high fraction of aromatic C such as humus, mature compost, and some biochars derived from N rich feedstocks, the limited accessibility to the organic C would slow down the expected rapid N mineralization because of the microbial use of the N from the decomposing materials.

The use of ^{13}C-CPMAS NMR allows a further clarification of the effects of different C types of OAs on N mineralization dynamics. However, when the ^{13}C-CPMAS NMR spectral regions were combined into synthetic indices from literature i.e. the alkyl C / O-alkyl-C and the carbonyl C / methoxyl C very poor relationships with N mineralization were found. The limited ability of ^{13}C-CPMAS NMR spectral index derived from the literature to explain ecological processes such as litter decay rate (Incerti et al. 2017), microbial

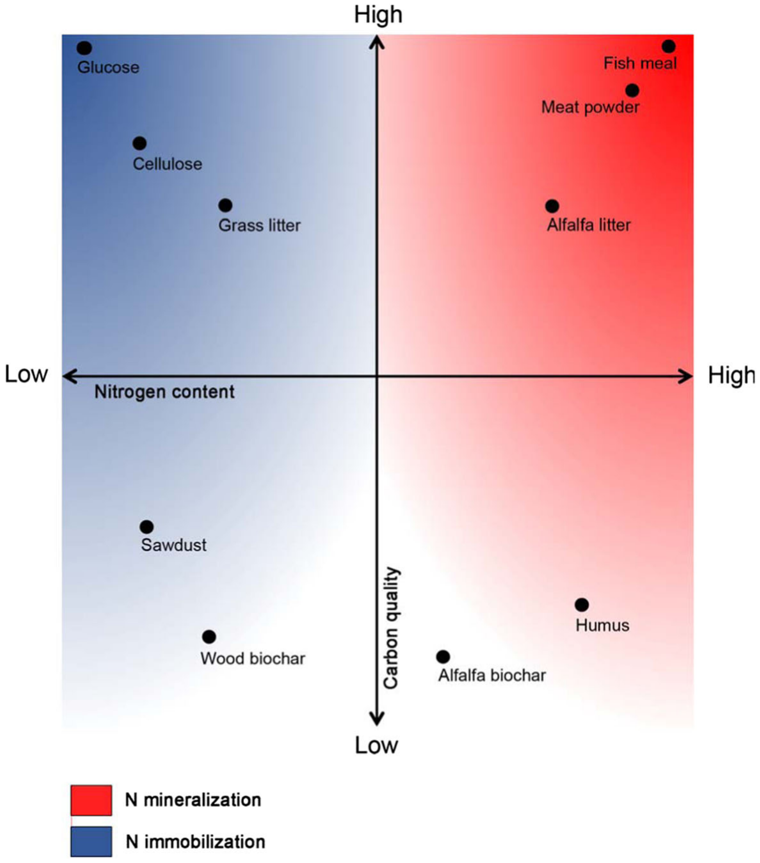

Fig. 7 Verbal-visual framework linking C quality of organic amendments to N mineralization. The framework predicts four main situations: i. fast N mineralization for organic amendments with high C quality and high N content (e.g. animal residues, N-fixing plant tissues); ii. slow N mineralization for organic amendments with low C quality but high N content (e.g. mature compost, humus extracts); iii. Slow, long-lasting N immobilization for organic amendments with low C quality and low N content (e.g., sawdust, wood and root residues, lignin rich plant residues, biochar); and; iv. rapid but short-term N immobilization for organic amendment with high C quality but low N content (e.g. simple sugars, cellulose rich but lignin poor plant residues)

and plant feeding preference (Bonanomi et al. 2017), soil water repellency (Cesarano et al. 2016) has been previously proven. Our correlation analysis applied to each 1 ppm-wide ^{13}C-NMR signal along the spectrum ($N = 200$) led to an improved identification of significant restricted spectral fractions. Specifically, we identified two restricted sections of the methoxyl C (50–57 ppm) and carbonyl C (172–180 ppm) regions highly correlated positively with soil NO_3^-. Moreover, we found that the restricted section of the di-O-alkyl C region (105–110 ppm) showed the highest, and most consistent in time, negative correlation with soil NO_3^-. These results were achieved by excluding poorly correlated sections from the corresponding wider ^{13}C-CPMAS NMR regions. In other words, the use of ^{13}C-CPMAS NMR regions from the literature, being based on wider signal ranges, included both informative (highly correlated, either positively or negatively) and useless (unrelated) signals to explain N mineralization. The reported extensive correlative analysis, however, is only a first step because the implications of our findings could be also relevant for agronomic applications. Additional work may further refine the information obtainable by restricted regions of the ^{13}C-CPMAS NMR spectra to provide a simple and easy to use new index capable of improving the prediction of N mineralization from different OAs without the known limitations of the C/N ratio.

In this regard, further studies will need to better understand the correlation shifts for most of the ^{13}C-CPMAS NMR regions during the period of incubation. For instance, an OA with high relative abundance of carbonyl C would release a large amount of NH_4^+ in the first phase of decomposition (100 days) but not afterwards. Concerning NO_3^-, amendment rich of methoxyl C would release large amount of mineral N in the medium and long-term. On the contrary, OA rich of both N and aromatic C, generated by either biological decomposition or pyrogenic processes, would not mineralize N in the short-term, but only after a long incubation period. This information would be the key to predict the patterns of N dynamics when different OAs are incorporated into soil.

Synthesis and conclusions

This study provided clear-cut evidence that the use of the C/N ratio as a predictor of N mineralization is limited to OAs mainly composed of easily accessible organic C but fails in case of OA rich in recalcitrant C (e.g., sawdust, biochar, humus extracts). Our work also highlighted the need to take into account the concept of C quality for better predictions of the N release pattern from a wide range of OA types. In this context, the use of ^{13}C-CPMAS NMR led to an improved description of OA quality. We identified three restricted sections of the ^{13}C-CPMAS NMR spectra highly correlated with N mineralization and immobilization. Future effort is still needed to fully explain the complex information contained in the ^{13}C-CPMAS NMR spectra into a user-friendly index for intelligible use by agronomic technicians and farmers. Here, we propose a verbal-visual framework where the C quality concept of OAs is explicitly presented and used to predict the N mineralization from a range of OA types (Fig. 7). This framework predicts four main situations: i. fast N mineralization for OAs with high C quality and high N content (e.g., animal residues, N-fixing leaf litter); ii. slow N mineralization for OAs with low C quality but high N content (e.g., very mature compost, humus extracts); iii. Slow, long-lasting N immobilization for OAs with low C quality and low N content (e.g., sawdust, wood and root residues, lignin rich leaf litter); and iv. rapid but short-term N immobilization for OA with high C quality but low N content (e.g., simple sugars, crop residues rich in cellulose but lignin poor). The challenge for future research will be to translate this verbal synthesis into a mathematical model capable of predicting the N mineralization dynamical patterns following OA application to the soil.

We are aware that our experiment was based on a limited number of OAs and only three soil types. In addition, our study was conducted under optimal conditions of temperature and soil moisture content. Consequently, further studies should investigate the consistency of results under more limiting conditions in terms both of temperature and soil moisture. Finally, a practical limit of our study might be related to the limited availability of solid state ^{13}C-CPMAS NMR spectroscopy, which is, so far, accessible to a limited number of laboratories. However, we are confident that this problem can be partially solved by making the data already obtained in our and other laboratories worldwide openly accessible by freely available data repository on the internet as is currently done for genomic and metagenomic libraries.

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