



## Interactive priming of soil N transformations from combining biochar and urea inputs: A $^{15}\text{N}$ isotope tracer study

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### ABSTRACT

Biochar has been found to interact with N transformations in soil but the mechanisms remain largely unknown. In this study we investigated the priming effect of combined biochar and urea inputs on soil inorganic N pools through an isotope tracer approach. Biochar was applied in combination with urea in two complementary laboratory experiments: (i) in the first one, three  $^{15}\text{N}$ -labeled organic amendments (wheat straw (WS)), its biochars produced at 350 °C (B350) and at 550 °C (B550) were added to soil in combination with unlabeled urea; (ii) in the second experiment the three same, but unlabeled, amendments were added to soil in combination with  $^{15}\text{N}$  labeled urea. This system allowed partitioning between three N sources: native soil N, biochar-derived N and urea-derived N. In addition,  $\text{CO}_2$  fluxes were measured to follow total C mineralization in soil and  $\text{N}_2\text{O}$  emissions were monitored. The proportion of N that mineralized from biochar was always below 0.5% of the added N. The co-addition of urea increased the concentration of  $\text{NH}_4^+$  derived from B350, but not from B550, demonstrating the lower mineralization of N in biochars produced at 550 °C. Whereas the addition of WS led to a rapid immobilization of N, we found that despite their high C:N, none of the biochars, applied at a rate of 1.5%, immobilized inorganic N in soil. On the contrary, significantly higher  $\text{NH}_4^+$  concentrations derived from native soil organic N (SON) and urea were found throughout the incubation when B550 was added. This effect can be attributed to an apparent priming effect since a net decrease in  $\text{CO}_2$  fluxes was recorded when biochar was added to the soil. The addition of glucose (a low molecular weight carbon source) stimulated an increase in  $\text{CO}_2$  fluxes in all treatments along with a net N immobilization in soil. However, both biochars significantly reduced C readily available to microbes, as proved by lower soil  $\text{CO}_2$  fluxes, and limited the immobilization of  $\text{NH}_4^+$  induced by glucose addition. Our results suggest that biochar may partially offset the mineralization of easily available organic C, buffering the immobilization of inorganic N in soil when labile organic compounds (e.g. root exudates, fresh manure, etc.) are incorporated.

### 1. Introduction

The interaction of biochar with soil N dynamics has attracted increasing attention over the last few years (Prommer et al., 2014; Hagemann et al., 2017; Zhou et al., 2017) and several reviews and meta-analyses have been published on the topic (Clough et al., 2013; Cayuela et al., 2015; Gul and Whalen, 2016; Nguyen et al., 2017). Essentially, biochar can alter microbiologically mediated N reactions, e.g.  $\text{N}_2$  fixation, mineralization of native soil organic N, nitrification and denitrification (Clough et al., 2013); but it can also physically adsorb

$\text{NH}_3$  (Taghizadeh-Toosi et al., 2012a) or release  $\text{NH}_4^+$  derived from its own mineralization (Schouten et al., 2012). Biochar has been found to influence soil N transformations, but the magnitude and direction of the effects are highly circumstantial and the mechanisms of interaction remain largely unknown (Jeffery et al., 2015). This is in part due to the challenge of tracing N compounds in soil, which rapidly change from one oxidation state to another. Many of these transformations imply the formation of gaseous products ( $\text{NH}_3$ , NO,  $\text{N}_2\text{O}$ ,  $\text{N}_2$ ), some of which are particularly difficult to measure (e.g.  $\text{N}_2$ ) (Groffman et al., 2006). Moreover, the high heterogeneity of biochars, the complexity of N

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reactions in soil and their dependence on specific soil characteristics hinder generalization.

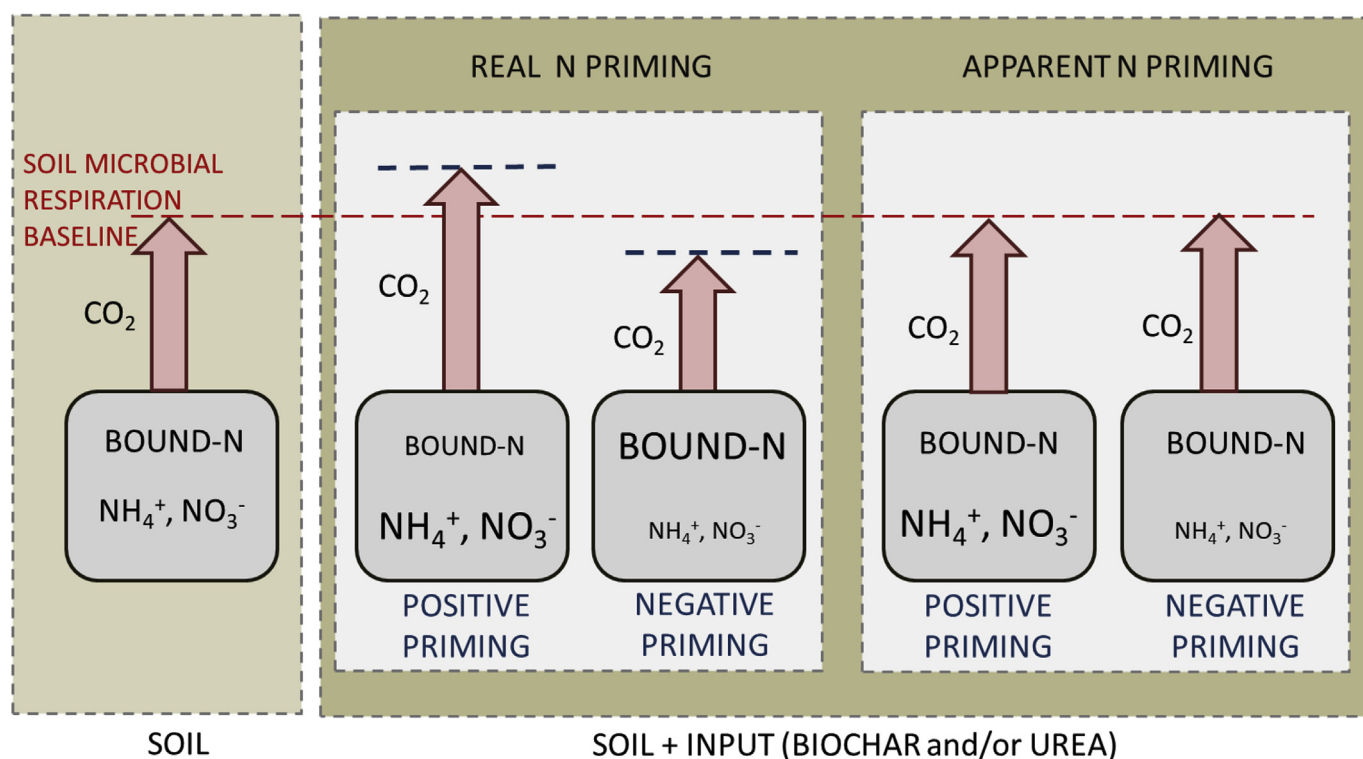
To date, most biochar studies have looked at the net changes in the extractable pool of inorganic N forms in soil (Nguyen et al., 2017). Only a few experiments analyzed the impact of biochar on gross N mineralization by the  $^{15}\text{N}$  isotope dilution technique (Cheng et al., 2012; Nelissen et al., 2012; Prommer et al., 2014; Mia et al., 2017) or the N use efficiency after the application of biochar in combination with  $^{15}\text{N}$  labeled mineral fertilizers (Hüppi et al., 2016; Zhou et al., 2017). To our knowledge there are no studies explicitly analyzing the impact of biochar on N priming in soil (also referred as “added N interaction” in older literature). Nitrogen priming is defined as the increase or decrease in the amount of native soil N released by microbial mineralization of soil organic matter caused by adding N compared to treatments without added N (Kuzakov et al., 2000; Liu et al., 2017). Nonetheless, as pointed by Kuzakov et al. (2000), the phenomenon is much broader and consists of a change (acceleration or deceleration) in the soil organic matter mineralization process through a “trigger” (which can be the addition of N, bioavailable C, rhizodeposition, mechanical disruption, as well as the addition of biochar). As pointed by Jenkinson et al. (1985), soil N priming can be “real” or “apparent” whose effect can be “positive” or “negative” (Azam, 2002) when an increase or a decrease of N inorganic pool occurs, respectively. Real N priming would occur when soil amendment/fertilization accelerates (or decelerates in the case of negative priming) the SON mineralization, which moves N into (or out of) the inorganic N pool. According to these authors, this phenomenon is necessarily accompanied by a net increase (or decrease) in  $\text{CO}_2$  fluxes (Fig. 1). “Apparent priming” occurs when soil amendment/fertilization does not affect SON mineralization and therefore the level of native organic N remains unchanged, but there is an increase (or decrease) in the native inorganic N pool (Fig. 1). This change in the inorganic N pool can be caused by pool substitution or isotope displacement reactions (Jenkinson et al., 1985; Azam, 2002). The concept

of “pool substitution” implies that labeled N added to a particular pool takes the place of unlabeled N that would otherwise have been removed from that pool (Jenkinson et al., 1985).

Whereas C priming is receiving increasing attention in biochar studies (Fang et al., 2015; Maestrini et al., 2015; Wang et al., 2016b; Cui et al., 2017), N priming, although suggested in field and laboratory experiments, has been far less investigated from a mechanistic point of view (Nelissen et al., 2012). This represents a weak point in terms of knowledge related to N cycling in soils, limiting the combined use of biochar and N fertilizers in agroecosystems.

In order to discriminate the different N-sources and identify (and quantify) N priming effects, the use of N isotope techniques is essential (Jenkinson et al., 1985; Barraclough, 1995). For instance, the use of artificially  $^{15}\text{N}$  labeled biomass has been used to follow the mineralization of N from residues, its interaction with native soil N (Azam et al., 1993), or its impact on fertilizer N availability (Gentile et al., 2008). However, the number of studies using  $^{15}\text{N}$  labeled biochar is still conspicuously low (Schouten et al., 2012; Jeffery et al., 2017) and we are not aware of studies partitioning three N sources (native soil N, biochar N and fertilizer N) in biochar studies. This limits our understanding on the interactions of biochar with both native N and added N fertilizers.

Some studies have reported decreased N mineralization or N immobilization (a negative priming effect) in soil after biochar amendment (Riaz et al., 2017; Wang et al., 2017), but this phenomenon has been generally attributed to the mineralization of less persistent C fractions in low-temperature, high C:N biochars (Clough et al., 2013; Budai et al., 2016) and it is generally a short-term effect (< 1 month) (Nguyen et al., 2017). On the contrary, high-temperature slow-pyrolysis biochars do not immobilize soil N (Case et al., 2015; Sánchez-García et al., 2016) and several studies have reported an increase in mineral N availability after their application in soil (Jeffery et al., 2017; Zhou et al., 2017). The mechanisms related to this enlarged mineral N pool



**Fig. 1.** Conceptual scheme depicting “real” and “apparent” N priming in soil after the addition of biochar/urea (based on Jenkinson et al., 1985). Bound-N refers to N that is not readily accessible to plants or to soil organisms (soil organic N (SON), microbial biomass-N or fixed  $\text{NH}_4^+$ ). The size of the letters symbolizes the size of the N pool. Positive priming implies a displacement from bound-N pool to the mineral N pool, whereas negative priming implies that less bound-N is released to the mineral pool. In apparent N priming, there is no change in the amount of bound-N in soil, in spite of the increase or decrease of the N mineral pool.

have been often speculated about, but rarely examined more closely. The additional inorganic N in soil could derive from (i) the mineralization of biochar itself (as shown by Jeffery et al. (2017)); (ii) by an increase in the mineralization of native soil organic N (SON), i.e. a positive priming effect; or (iii) by improved N retention mechanisms, which could be biotic (e.g. decrease in denitrification) or abiotic (e.g. adsorption or physical protection). For instance, Maestrini et al. (2014) found that biochar had a positive priming effect on SOM mineralization with a parallel increase in  $\text{NH}_4^+$  concentration in soil that they related to an increased gross N mineralization. However, other studies have reported a decrease in gross N mineralization with biochar (Prommer et al., 2014; Mia et al., 2017), which shows that the direction of the priming effect (positive or negative) probably depends on biochar properties as well as N availability in soil.

Therefore, we investigated the mechanisms by which biochar influences soil native N and added N transformations. We hypothesized that: (i) the amount of N mineralized from high temperature slow pyrolysis biochar is negligible and that the addition of an external N source would increase N mineralization from uncharred organic matter and low-temperature biochar, but not from high-temperature biochar; (ii) whereas low-temperature biochar and uncharred organic matter might induce N immobilization in soil, high-temperature biochar would not and (iii) when extra N is added, uncharred organic matter and low-temperature biochar might induce a positive priming effect on soil native N, but high-temperature biochar would not.

## 2. Materials and methods

### 2.1. Soil and amendments used for incubations

A loam soil (Typic Dystrudept; USDA, 1999) was collected from the 0–0.30 m layer of a private field previously under corn (*Zea mays* L.) in North-East Italy (46°08'01.9" N 13°13'44.6" E). The soil was air dried and sieved at 2 mm before chemical characterization and use. The main soil chemical and physical properties are reported in Table 1.

Six organic amendments were used for the incubations:

1. Wheat straw (WS)
2.  $^{15}\text{N}$  labeled wheat straw ( $^{15}\text{N}$ -WS).
3. Biochar produced from WS by slow pyrolysis at highest heating temperature (HHT) of 350 °C (B350)
4. Biochar produced from WS by slow pyrolysis at HHT of 550 °C (B550)
5.  $^{15}\text{N}$  labeled biochar produced from  $^{15}\text{N}$ -WS by slow pyrolysis at HHT of 350 °C ( $^{15}\text{N}$ -B350)
6.  $^{15}\text{N}$  labeled biochar produced from  $^{15}\text{N}$ -WS by slow pyrolysis at HHT of 550 °C ( $^{15}\text{N}$ -B550)

**Table 1**  
Soil main chemical-physical properties

| Variable                     | units                     | value  |
|------------------------------|---------------------------|--------|
| Physical Properties          |                           |        |
| Clay                         | g (100 g) <sup>-1</sup>   | 19.6   |
| Silt                         | g (100 g) <sup>-1</sup>   | 31.9   |
| Sand                         | g (100 g) <sup>-1</sup>   | 48.5   |
| Bulk Density                 | g cm <sup>-3</sup>        | 1.32   |
| Chemical Properties          |                           |        |
| $\text{NH}_4^+$ -N           | mg kg <sup>-1</sup>       | 17.4   |
| $\text{NO}_3^-$ -N           | mg kg <sup>-1</sup>       | 4.3    |
| $\text{PO}_4^{3-}$ -P        | mg g <sup>-1</sup>        | 1.1    |
| Organic C                    | g (100 g) <sup>-1</sup>   | 1.383  |
| Organic N                    | g (100 g) <sup>-1</sup>   | 0.16   |
| $^{15}\text{N}$ abundance    | at-%                      | 0.3517 |
| Cation Exchange Capacity     | meq (100 g) <sup>-1</sup> | 15.09  |
| $\text{P}_2\text{O}_5$ Olsen | mg kg <sup>-1</sup>       | 20     |
| pH                           |                           | 5.4    |

The preparation of both labeled and unlabeled organic amendments was as follows: wheat plants (*Triticum aestivum* L.) were grown in six 50-L pots. One half of the pots received fertilizer with natural  $^{15}\text{N}$  abundance (0.37 atom%), while the remaining pots were fertilized with a solution enriched in  $^{15}\text{N}$  (60 atom%), both at a rate of 50 mg N kg<sup>-1</sup> soil. WS and  $^{15}\text{N}$ -WS consisted of stems, leaves and chaff and were collected at physiological maturity, washed with deionized water, dried at 65 °C to constant weight, and ground to < 1 mm before chemical analyses and use. Four biochars were produced by slow pyrolysis from WS and  $^{15}\text{N}$ -WS as described by Enders et al. (2012) at two HHTs: 350 and 550 °C. The main chemical and physical characteristics of the amendments are shown in Table 2.

### 2.2. Experimental set up

A dual-isotope approach ( $^{14}\text{N}$ ,  $^{15}\text{N}$ ) was used (Gentile et al., 2008) that allows partitioning between three N sources (native soil N, biochar N and urea N). Two identical parallel incubation experiments (except for the different isotopic signatures of the inputs) were carried out at a constant temperature of 27 °C and controlled moisture (see below the different phases of the incubation for details). In experiment 1 we added naturally labeled biomass (WS, B350 and B550) in combination with  $^{15}\text{N}$  labeled urea. In experiment 2 we added  $^{15}\text{N}$  labeled biomass ( $^{15}\text{N}$ -WS,  $^{15}\text{N}$ -B350 and  $^{15}\text{N}$ -B550) in combination with unlabeled urea (see Table 3).

Experimental units consisted of 50 g soil (oven dry weight basis) in 100-mL polypropylene jars. The wheat straw and biochars application rate was 1.5 g 98.5 g<sup>-1</sup> DM soil (Cayuela et al., 2013) corresponding to 0.62, 0.90 and 1.03 g C 100 g<sup>-1</sup> soil for WS (or  $^{15}\text{N}$ -WS), BC350 (or  $^{15}\text{N}$ -B350) and BC550 (or  $^{15}\text{N}$ -B550), respectively. The experiment was laid out as a completely randomized design with four replicates per treatment for gas measurements and three replicates per treatment for destructive soil sampling to determine mineral N.

Each experiment lasted for 42 days and consisted of three phases, each phase comprising 14 days (Table 3):

- In **phase 1** (first 14 days of incubation) the moisture was kept constant at 60% of the water filled pore space (WFPS) in order to provide optimal conditions for mineralization. The main objective in this phase was to study the impact of wheat straw and biochars on N priming (soil native N mineralization) and to follow the mineralization of N from wheat straw and biochars.
- In **phase 2** (days 15–28) urea (application rate 51 mg N kg<sup>-1</sup> soil) was added to the experimental units from phase 1. A volume of 11.4 mL of a solution containing 0.223 mg N mL<sup>-1</sup> was added to each experimental unit, which temporarily increased the WFPS to 90%. After 10 days, moisture decreased to 60% WFPS again, which was kept constant until the end of this phase. Treatments in experiment 1 were fertilized with  $^{15}\text{N}$  labeled (98 atom%) urea, whereas the treatments in experiment 2 were fertilized with unlabeled urea. The aim of this phase was to investigate the interactive priming effects of urea and biochar on soil native N mineralization, the impact of urea on biochar N mineralization and the impact of biochar on urea N mineralization.
- In **phase 3** (days 28–42) glucose was added (0.7 mg C g soil<sup>-1</sup>) to the experimental units from phase 2. A volume of 11.8 mL of a solution containing 2.96 mg C mL<sup>-1</sup> was added to each experimental unit increasing WFPS to 90%. The main goal was increasing microbial activity and assessing the interactive priming effect of biochar and urea when there was a readily available C source.

A complete overview of the treatments, experimental conditions and timing during the incubation is provided in Table 3.

**Table 2**  
Main chemical properties of the <sup>15</sup>N labeled and not labeled wheat straw and biochars.

| Amendments           | C    | H   | N    | O    | <sup>15</sup> N | C:N                      | O:C  | H:C  | Water | Volatile Matter | Fixed Carbon | Ash | Yield |
|----------------------|------|-----|------|------|-----------------|--------------------------|------|------|-------|-----------------|--------------|-----|-------|
|                      | %    |     |      |      | at-%            | (mol mol <sup>-1</sup> ) |      |      | %     |                 |              |     |       |
| WS                   | 41.5 | 5.7 | 0.73 | 51.8 | 0.4392          | 57                       | 1.25 | 1.64 | 4.8   | 70.8            | 17.0         | 0.4 | –     |
| B350                 | 61.9 | 3.9 | 1.32 | 32.7 | 0.4263          | 47                       | 0.53 | 0.75 | 4.3   | 29.1            | 50.4         | 1.2 | 42.6  |
| B550                 | 66.7 | 2.1 | 1.29 | 29.8 | 0.4280          | 52                       | 0.45 | 0.38 | 3.9   | 6.5             | 68.9         | 1.0 | 31.9  |
| <sup>15</sup> N-WS   | 41.2 | 5.7 | 0.71 | 52.1 | 29.5763         | 58                       | 1.26 | 1.65 | 6.1   | 71.6            | 19.1         | 0.1 | –     |
| <sup>15</sup> N-B350 | 58.3 | 3.7 | 1.38 | 36.4 | 34.4640         | 42                       | 0.62 | 0.76 | 4.4   | 28.3            | 50.7         | 1.3 | 40.4  |
| <sup>15</sup> N-B550 | 70.2 | 2.0 | 1.06 | 26.6 | 36.7378         | 66                       | 0.38 | 0.34 | 2.3   | 5.3             | 73.5         | 1.5 | 30.9  |

WS: wheat straw; B350: biochar produced from WS by slow pyrolysis at the highest heating temperature (HHT) of 350 °C; B550: biochar produced from WS by slow pyrolysis at HHT of 550 °C.

2.3. Measurements

2.3.1. CO<sub>2</sub> and N<sub>2</sub>O emissions

Fluxes of CO<sub>2</sub> and N<sub>2</sub>O were measured 19 times over a period of 42 days. Fluxes were more frequently measured during the initial 5 days of each phase, decreasing the frequency to once a week subsequently. For gas sampling each unit was sealed with gas-tight polypropylene screw caps for an accumulation period of 60 min. The headspace gas was then sampled directly with a membrane air pump (Optimal 250, Schego, Offenbach am Main, Germany), attached to a gas chromatograph (VARIAN CP-4900 Micro-GC, Palo Alto, CA, USA) (Mondini et al., 2010).

2.3.2. Mineral nitrogen

Parallel soil incubations were set up for each destructive soil sampling (3 replicates per treatment). Soil extractions were carried out at days 4, 14, 18, 28, 32 and 42. Soils were thoroughly homogenized before sampling. Moist soil (1:10 w:v, dry weight basis) was shaken with 0.01 M CaCl<sub>2</sub> for 2 h and then centrifuged (15 min, 2509 G). Extracts were filtered at 0.45 μm before analyses. NH<sub>4</sub><sup>+</sup> was determined by a colorimetric method based on Berthelot's reaction. NO<sub>3</sub><sup>-</sup> and NO<sub>2</sub><sup>-</sup> were determined by ion chromatography (HPLC, model 861, Metrohm AG, Herisau, Switzerland).

2.3.3. Isotopic signature of N in soil and amendments

The <sup>15</sup>N enrichment of soil mineral N was determined according to a micro-diffusion technique based on Ros et al. (2010). The principle of

this technique is to allow the N pool of interest from soil extracts to be fixed in acid traps consisting in Glass fiber microfilters (GF/A, 6 mm diameter) spiked with 2M KHSO<sub>4</sub> and sealed with Teflon tape. The required amount of soil extracts containing 30–100 μg of NH<sub>4</sub><sup>+</sup> or NO<sub>3</sub><sup>-</sup> was collected in 100-mL plastic air-tight containers and added with KCl to get a 1M KCl solution, leaving similar headspaces in each container. Both NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> were recovered in acid traps by a two-step procedure. NH<sub>4</sub><sup>+</sup> was recovered rising pH up to 10 with 0.4 g of MgO, and leaving one trap in a closed container for 7 days at 25 °C with periodical shaking. NO<sub>3</sub><sup>-</sup> was recovered from the same solutions converting NO<sub>3</sub><sup>-</sup> to NH<sub>4</sub><sup>+</sup> by adding 0.4 g of Devarda's alloy and leaving a new trap in a closed container for 7 days. The sealed filters containing NH<sub>4</sub><sup>+</sup> or NO<sub>3</sub><sup>-</sup> were collected, washed with deionized water, unsealed, dried and packed in tin capsules for total N and <sup>15</sup>N enrichment analysis.

Filters from micro-diffusion as well as dry soil, straw and biochars were analyzed using an automated C/N analyzer-isotope ratio mass spectrometer PDZ Europa ANCAGSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK).

2.4. Data analysis

2.4.1. Mineral N derived from wheat straw, biochars or urea

The <sup>15</sup>N enrichment of the different inputs (wheat straw, biochars and urea) allowed for direct calculation of WS, biochar or urea derived-N using the following equation (Gentile et al., 2008):

**Table 3**

Details of the experiments, including the three phases of the incubation, the soil moisture pattern (WFPS), the different treatments, and the timing of the measurements (mineral N and gas fluxes).

|                         | Amendment addition   | Phase 1 (amendment) (days)            |    |    |    |    | Phase 2 (urea addition) (days) |    |    |    |    | Phase 3 (glucose addition) (days) |    |    |    |    |
|-------------------------|--|---------------------------------------|----|----|----|----|--------------------------------|----|----|----|----|-----------------------------------|----|----|----|----|
|                         |  | 0                                     | 1  | 2  | 4  | 14 | 15                             | 16 | 17 | 18 | 28 | 29                                | 30 | 31 | 32 | 42 |
| Experimental Conditions | x  |                                       |    |    |    |    | x                              |    |    |    |    |                                   |    |    |    |    |
|                         | Urea addition  |                                       |    |    |    |    | x                              |    |    |    |    |                                   |    |    |    |    |
|                         | Glucose addition   |                                       |    |    |    |    |                                |    |    |    |    | x                                 |    |    |    |    |
|                         | WFPS (%)   | 60                                    | 60 | 60 | 60 | 60 | 90                             | –  | –  | –  | 60 | 90                                | –  | –  | –  | 60 |
| Experiment 1            | Treatment 1  | WS (Wheat straw)                      |    |    |    |    | WS - <sup>15</sup> N-UR        |    |    |    |    | WS - <sup>15</sup> N-UR-GLU       |    |    |    |    |
|                         | Treatment 2  | B350 (Biochar 350 °C)                 |    |    |    |    | B350 - <sup>15</sup> N-UR      |    |    |    |    | B350 - <sup>15</sup> N-UR-GLU     |    |    |    |    |
|                         | Treatment 3  | B550 (Biochar 550 °C)                 |    |    |    |    | B550 - <sup>15</sup> N-UR      |    |    |    |    | B550 - <sup>15</sup> N-UR-GLU     |    |    |    |    |
|                         | Treatment 4  | NoF                                   |    |    |    |    | <sup>15</sup> N-UR             |    |    |    |    | <sup>15</sup> N-UR-GLU            |    |    |    |    |
| Experiment 2            | Treatment 1  | <sup>15</sup> N WS                    |    |    |    |    | <sup>15</sup> N WS - UR        |    |    |    |    | <sup>15</sup> N WS - UR -GLU      |    |    |    |    |
|                         | Treatment 2  | <sup>15</sup> N B350 (Biochar 350 °C) |    |    |    |    | <sup>15</sup> N BC350 - UR     |    |    |    |    | <sup>15</sup> N B350-UR-GLU       |    |    |    |    |
|                         | Treatment 3  | <sup>15</sup> N B550 (Biochar 550 °C) |    |    |    |    | <sup>15</sup> N BC550 - UR     |    |    |    |    | <sup>15</sup> N B550-UR-GLU       |    |    |    |    |
|                         | Treatment 4  | NoF                                   |    |    |    |    | NoF                            |    |    |    |    | NoF-GLU                           |    |    |    |    |
| Measurements            | Soil NH <sub>4</sub> <sup>+</sup> and NO <sub>3</sub> <sup>-</sup> | x                                     |    |    |    |    | x                              |    |    |    |    | x                                 |    |    |    |    |
|                         | N <sub>2</sub> O and CO <sub>2</sub>                               | x                                     | x  | x  | x  | x  | x                              | x  | x  | x  | x  | x                                 | x  | x  | x  | x  |

WS: wheat straw; B350: biochar produced from WS by slow pyrolysis at the highest heating temperature (HHT) of 350 °C; B550: biochar produced from WS by slow pyrolysis at HHT of 550 °C. UR: urea; NoF: not fertilized soil. GLU: glucose. The three phases of the incubation are defined by: (i) phase 1: addition of amendments (1.5% dry weight of WS, B350 or B550) at day zero, (ii) phase 2: addition of urea (51 mg N kg<sup>-1</sup> soil) at day 15, (iii) phase 3: addition of glucose (0.7 mg C g soil<sup>-1</sup>) at day 29. <sup>15</sup>N refers to amendments or urea enriched with <sup>15</sup>N.



$$N_{input} = N_{sample} \left[ \frac{15N_{sample} - 15N_{natural\ abundance}}{15N_{input} - 15N_{natural\ abundance}} \right] \quad (1)$$

where  $N_{input}$  = quantity of N derived from the input,  $N_{sample}$  = N measured in the sample,  $^{15}N_{sample}$  = atom % measured in the sample,  $^{15}N_{natural\ abundance}$  = atom% of the corresponding soil control, and  $^{15}N_{input}$  = atom% of the input.

Thus, experiment 1 (with unlabeled urea) was used to calculate biochar (or wheat straw) derived N and experiment 2 (with labeled urea) was used to calculate urea-derived N. Soil native N was calculated as the difference between the total mineral N measured and the mineral N of input-derived N.

The priming effect (PE) was calculated as the change of the mineral N produced from SOM (Kuzuyakov et al., 2000; Conrad et al., 2012; Ye et al., 2016; Zhu et al., 2016) caused by soil amendment/fertilization (e.g. straw, biochar, urea or a combination of amendments and urea). Mineral N derived from SOM ( $N_{som}$ ) turnover without priming effect was measured in non-amended soils. On the other hand, in amended/fertilized soils, mineral N production from SOM ( $N_{som}^*$ ) was calculated by the difference between total mineral N and mineral N derived from amendments/fertilizers (see Eq. (1)) as follows:

$$N_{som}^* = N_{sample} - N_{input} \quad (2)$$

Where  $N_{som}^*$  = quantity of N derived from SOM degradation in amended/fertilized soil;  $N_{sample}$  = total mineral N measured in fertilized/amended soils;  $N_{input}$  = quantity of mineral N derived from amendments and/or urea.

The priming effect (PE), being the variation in mineral N derived from SOM degradation, was calculated as follows:

$$PE = N_{som}^* - N_{som} \quad (3)$$

Where PE = priming effect expressed in  $mg\ kg^{-1}$  of mineral nitrogen;  $N_{som}^*$  and  $N_{som}$  as defined above.

$$PE^* = \frac{N_{som}^*}{N_{som}} - 1 \quad (4)$$

Where  $PE^*$  = priming effect expressed as a fraction of mineral N from SOM degradation in not amended/fertilized control;  $N_{som}^*$  and  $N_{som}$  as defined above.

#### 2.4.2. Gas fluxes calculation

Gas fluxes were calculated assuming a linear increase during the accumulation (closed) period, an approach that was verified prior to the experiments. Cumulative  $CO_2$  and  $N_2O$  emissions were calculated assuming linear changes in fluxes between adjacent measurement points. Cumulative fluxes were plotted as a proportion of added C (with amendments and glucose) for  $CO_2$ -C and as  $N_2O$ -N  $kg^{-1}$  soil.

#### 2.4.3. Statistical analyses

Mineral N data were subjected to an analysis of variance (ANOVA) using a general linear model (SPSS version 17.0, Chicago, Illinois). Within each incubation phase the combination amendment by sampling date was considered as a single treatment. Means were separated according to the LSD Sidak test with  $p < 0.05$ . Normality of distribution and homogeneity of variance were verified using the Kolmogorov–Smirnov and Levene tests, respectively. Data are presented as mean values of three replicates with relative standard errors, except for gas measurements, where data represent the mean of four replicates.

### 3. Results

#### 3.1. Mineralization of N from biochars or wheat straw

As measured by isotopic analysis, the proportion of N that mineralized from biochars or wheat straw was very low (Table 4),

representing less than 0.3% of the added N during phases 1 and 3 and less than 3% of the added N during phase 2 (Table 4b). The addition of urea (phase 2) increased the mineralization of B350-N and specially WS-N, whereas inorganic N originating from B550 remained largely constant at about 0.1% even after urea addition. Glucose addition (phase 3) led to a significant mineral N immobilization for all treatments (Fig. S1), including the amendment-derived-N.

As hypothesized, the amendment-derived-N declined in the order: WS > BC350 > BC550 throughout the three phases, which is in accordance with C mineralization data (Table 6).

The inorganic N originating from the amendments represented only a small fraction of total mineral N in soil (Table 4c), with values lower than 2% in the case of biochars and 12% for WS.

#### 3.2. Priming effect (PE) of biochar on native soil N

Fig. 2 shows native mineral N concentration in soil during the course of the incubation, i.e. how N originally present in soil was affected by the different amendments. In Table 5 we report PE calculations according to equations (3) and (4) (see materials and methods section). The addition of B550 in phase 1 (Fig. 2a) had a positive priming effect on soil N (17% and 14% increase in total mineral N from SOM degradation in the first and second sampling dates, respectively), since the concentration of soil native  $NH_4^+$ -N in B550 was significantly higher than in the control at days 4 and 14. There was a positive priming effect (Table 5) for B350 treatment as well (7% and 6% increase in total mineral N from SOM degradation in the first and second sampling dates, respectively), although only significant at day 14. Soil native  $NO_3^-$ -N concentration was less affected by biochar addition and only B350 displayed a significantly lower  $NO_3^-$ -N concentration at day 14 compared to the control (NoF).

In phase 2, the addition of urea alone significantly increased soil native  $NH_4^+$ -N and  $NO_3^-$ -N concentrations (Fig. 2b) compared to the NoF treatment, corresponding to a positive PE of 20% and 30% at day 18 and 28, respectively (Table 5). We found a significant interaction when urea was added in combination with biochar, since both B350 and B550 additions (i) increased the positive priming effect of urea on soil native  $NH_4^+$ -N, and (ii) offset the positive priming effect of urea on native  $NO_3^-$ -N at day 18. However, this interaction did not modify the total mineral N compared to the UR treatment and was short-term, since it was not detected at day 28.

Glucose addition (Fig. 2c) decreased soil native  $NH_4^+$ -N and  $NO_3^-$ -N concentrations at day 32 with respect to day 28 in all treatments. However, we found that biochar consistently counteracted the  $NH_4^+$ -N immobilization prompted by glucose addition. As a consequence soil native  $NH_4^+$ -N concentrations in biochar amended soils (16 and  $18\ \mu g\ g^{-1}$  soil, on the average at days 32 and 42, respectively) were significantly higher than in the urea-only treatment (12 and  $13\ \mu g\ g^{-1}$  soil at days 32 and 42, respectively). This pattern was not observed for  $NO_3^-$ -N. On the whole, in glucose amended soils, PE due to urea fertilization was increased when biochar was added (Table 5).

The addition of WS led to a strong immobilization of soil native N (both  $NH_4^+$ -N and  $NO_3^-$ -N) throughout the three phases of the incubation.

#### 3.3. Impact of biochar on urea-N turnover

Fig. 3 shows urea-derived mineral N concentrations in soil during the course of the incubation, i.e. how the different treatments affected urea-N turnover in soil. Similar to native soil N, we found an interactive effect between biochar and urea, since shortly after fertilization B550 significantly increased urea-derived  $NH_4^+$ -N (Fig. 3a) with respect to addition of only urea (day 18). This effect did not persist at day 28. None of the biochars had a significant impact on urea-derived  $NO_3^-$ -N concentrations in soil.

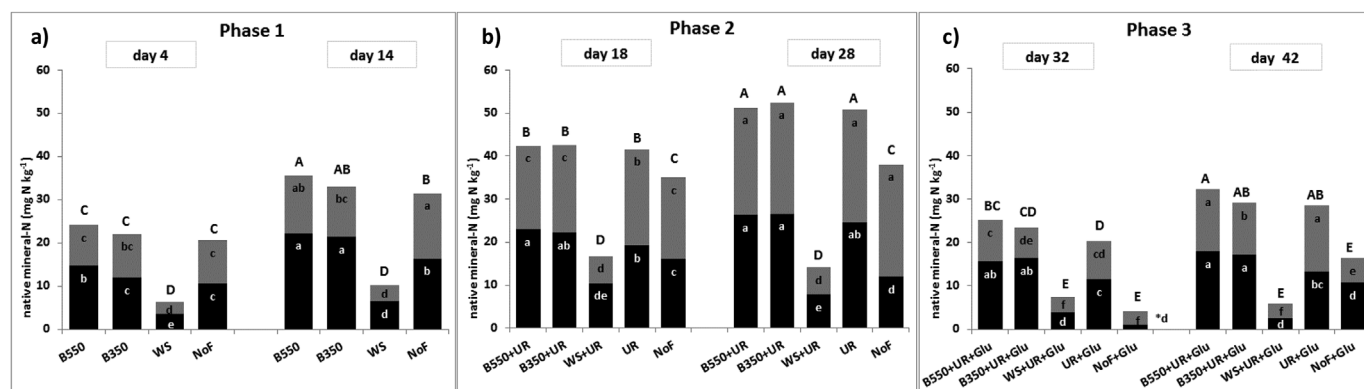
Addition of glucose immobilized urea-derived  $NH_4^+$ -N (Fig. 3b). We

**Table 4**

Inorganic N (NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N) in soil derived from the mineralization of biochars or wheat straw as deduced from the isotopic dilution and expressed as: (a) mg N kg<sup>-1</sup> soil; (b) relative to N added with the amendment (WS, B350 or B550); (c) relative to the total inorganic N extracted from soil.

|   | Phase 1 |        | Phase 2 |        |        |        | Phase 3 |    |      |    |      |     |
|---|---------|--------|---------|--------|--------|--------|---------|----|------|----|------|-----|
|   | Day 4   | Day 14 | Day 18  | Day 28 | Day 32 | Day 42 |         |    |      |    |      |     |
| <b>(a) amendment derived mineral N (mg N kg<sup>-1</sup>)</b>   |         |        |         |        |        |        |         |    |      |    |      |     |
| B550  | 0.15    | Aa     | 0.20    | Aa     | 0.19   | Ca     | 0.25    | Ca | 0.12 | Aa | 0.16 | Ba  |
| B350  | 0.35    | Abc    | 0.54    | Abc    | 0.68   | Bab    | 0.94    | Ba | 0.33 | Ac | 0.59 | Abc |
| WS  | 0.31    | Ac     | 0.17    | Bc     | 2.94   | Aa     | 2.09    | Ab | 0.24 | Ac | 0.10 | Bc  |
| <b>(b) amendment derived mineral N (% added N)</b>              |         |        |         |        |        |        |         |    |      |    |      |     |
| B550  | 0.09    | Aa     | 0.13    | Aa     | 0.13   | Ba     | 0.16    | Ba | 0.07 | Aa | 0.09 | Aa  |
| B350  | 0.17    | Aa     | 0.26    | Aa     | 0.34   | Ba     | 0.46    | Ba | 0.16 | Aa | 0.30 | Aa  |
| WS  | 0.29    | Ac     | 0.16    | Ac     | 2.78   | Aa     | 1.97    | Ab | 0.22 | Ac | 0.08 | Ac  |
| <b>(c) amendment derived mineral N/total mineral N soil (%)</b> |         |        |         |        |        |        |         |    |      |    |      |     |
| B550  | 0.61    | Ca     | 0.57    | Ba     | 0.22   | Ba     | 0.28    | Ba | 0.31 | Ba | 0.33 | Ca  |
| B350  | 1.55    | Ba     | 1.60    | Aa     | 0.79   | Ba     | 1.02    | Ba | 0.89 | Ba | 1.24 | Ba  |
| WS  | 4.82    | Ac     | 1.65    | Ae     | 10.43  | Ab     | 11.87   | Aa | 2.98 | Ad | 2.51 | Ad  |

WS: wheat straw; B350: biochar produced from WS at the highest heating temperature (HHT) of 350 °C; B550: biochar produced from WS at HHT of 550 °C. See Table 3 for the description of the three phases. The identification of different groups was carried out considering as factors: amendment (capital letters) and time of incubation (small letters) according to the LSD Sidak test (*P* < 0.05).



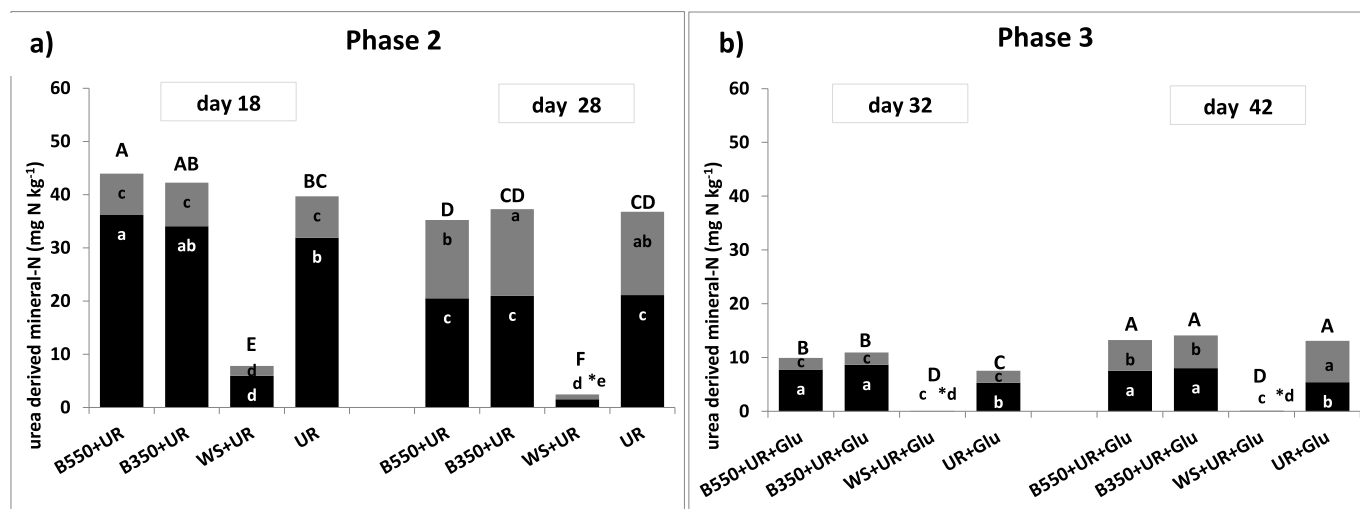
**Fig. 2.** Soil native mineral-N measured during the incubation as NH<sub>4</sub><sup>+</sup>-N (black bars) and NO<sub>3</sub><sup>-</sup>-N (grey bars). WS: soil amended with wheat straw; B350: soil amended with biochar produced from WS by slow pyrolysis at the highest heating temperature (HHT) of 350 °C; B550: soil amended with biochar produced from WS by slow pyrolysis at HHT of 550 °C; UR: urea; NoF: not fertilized soil; GLU: glucose. See Table 3 for the description of the three phases. Within each phase, bars with the same letters are not significantly different according to the LSD Sidak test (*p* < 0.05). Capital letters refer to the sum of NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N. \* indicates NH<sub>4</sub><sup>+</sup>-N when necessary.

**Table 5**

Priming effect (± standard error) measured on the total mineral N pool and expressed as: a) mg N kg<sup>-1</sup> soil; (b) relative to mineral N derived from SOM mineralization.

|  | Phase 1 |        | Phase 2 |        |        |        | Phase 3 |        |      |        |      |        |
|--|---------|--------|---------|--------|--------|--------|---------|--------|------|--------|------|--------|
|  | day 4   | day 14 | day 18  | day 28 | day 32 | day 42 |         |        |      |        |      |        |
| <b>a) PE (mg mineral N kg<sup>-1</sup> soil)</b> |         |        |         |        |        |        |         |        |      |        |      |        |
| B550   | 3.5     | ± 1.6  | 4.1     | ± 1.4  | 7.4    | ± 1.2  | 13.2    | ± 1.0  | 21.2 | ± 2.0  | 24.4 | ± 1.6  |
| B350   | 1.4     | ± 2.7  | 1.5     | ± 0.1  | 9.4    | ± 0.9  | 14.4    | ± 1.0  | 19.7 | ± 1.4  | 22.3 | ± 1.2  |
| WS   | -14.3   | ± 1.1  | -21.3   | ± 0.5  | -18.2  | ± 1.0  | -23.9   | ± 1.2  | 3.4  | ± 1.5  | -2.7 | ± 0.9  |
| UR   |         |        | 6.7     | ± 1.2  | 12.8   | ± 1.4  | 16.2    | ± 1.8  | 19.8 | ± 0.6  |      |        |
| <b>b) PE (fraction)</b>                          |         |        |         |        |        |        |         |        |      |        |      |        |
| B550   | 0.17    | ± 0.06 | 0.14    | ± 0.08 | 0.2    | ± 0.01 | 0.4     | ± 0.01 | 6.0  | ± 0.57 | 3.3  | ± 0.47 |
| B350   | 0.07    | ± 0.12 | 0.06    | ± 0.02 | 0.3    | ± 0.06 | 0.4     | ± 0.05 | 5.5  | ± 0.38 | 3.0  | ± 0.31 |
| WS   | -0.69   | ± 0.05 | -0.67   | ± 0.03 | -0.5   | ± 0.02 | -0.7    | ± 0.02 | 1.1  | ± 0.62 | -0.5 | ± 0.10 |
| UR   |         |        | 0.2     | ± 0.01 | 0.3    | ± 0.03 | 4.0     | ± 0.38 | 2.5  | ± 0.05 |      |        |

WS: wheat straw; B350: biochar produced from WS at the highest heating temperature (HHT) of 350°C; B550: biochar produced from WS at HHT of 550 °C. See Table 3 for the description of the three phases.



**Fig. 3.** Urea-derived mineral-N measured during the incubation as NH<sub>4</sub><sup>+</sup>-N (black bars) and NO<sub>3</sub><sup>-</sup>-N (grey bars). **WS:** soil amended with wheat straw; **B350:** soil amended with biochar produced from WS by slow pyrolysis at the highest heating temperature (HHT) of 350 °C; **B550:** soil amended with biochar produced from WS by slow pyrolysis at HHT of 550 °C. **UR:** soil fertilized with urea only; **Control:** not fertilized soil; **GLU:** glucose. See Table 3 for the description of the three phases. Within each phase, bars with the same letters are not significantly different according to the LSD Sidak test (*p* < 0.05). Capital letters refer to the sum of NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N. \* indicates NH<sub>4</sub><sup>+</sup>-N when necessary.

observed a similar pattern as with native NH<sub>4</sub><sup>+</sup>-N in phase 3, i.e. both biochars offset the NH<sub>4</sub><sup>+</sup>-N immobilization caused by glucose addition. With WS additions, urea derived mineral N after glucose addition was close to zero at both sampling dates.

### 3.4. CO<sub>2</sub> and N<sub>2</sub>O fluxes

As expected, WS addition led to the highest CO<sub>2</sub>-C fluxes (Fig. 4a, Table 6), with cumulative emissions of 4052 mg C kg<sup>-1</sup> soil after 42 days. The extra cumulative CO<sub>2</sub> flux with additions of WS at the end of the incubation (cumulative CO<sub>2</sub>-C from WS treatment minus cumulative CO<sub>2</sub>-C from urea treatment) corresponds to 49% of the added C with WS. Conversely, biochar treatments emitted the same amount of CO<sub>2</sub> as the urea treatment.

The addition of glucose at day 29 produced a sharp increase in CO<sub>2</sub> emissions in all treatments (Fig. 4a). However, it is worth mentioning that both biochars reduced cumulative CO<sub>2</sub>-C recorded 14 days after glucose addition compared to the urea treatment (518 and 520 mg C kg<sup>-1</sup> soil<sup>-1</sup> for B550 and B350 compared to 701 mg C kg<sup>-1</sup> soil<sup>-1</sup> in

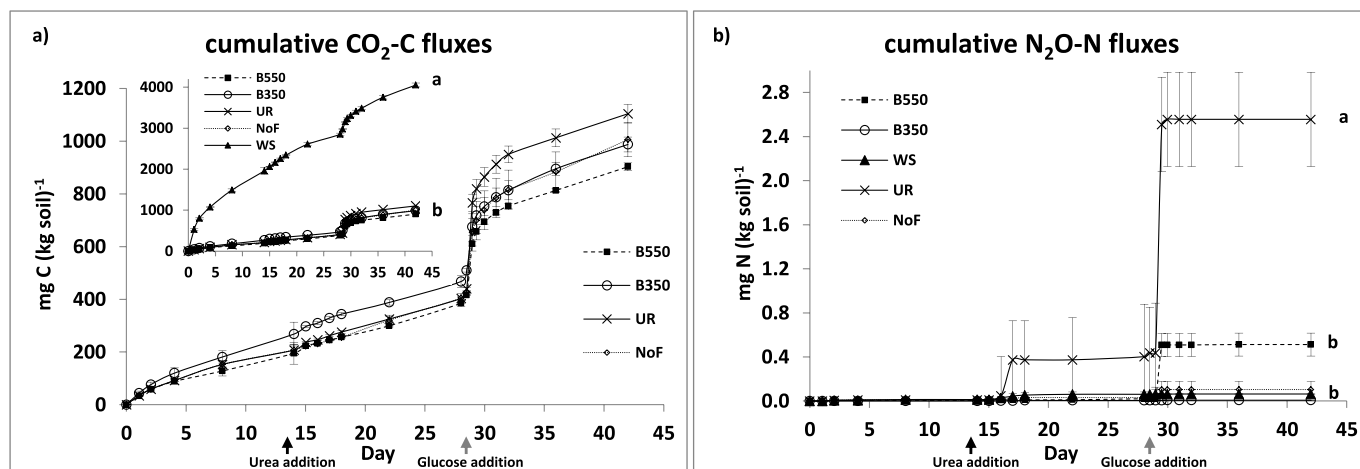
the urea treatment).

Total N<sub>2</sub>O emissions were generally low (below 2.5 mg N kg soil<sup>-1</sup>) throughout the incubation (Fig. 4b). The addition of urea in phase 2 and the subsequent addition of glucose and increased WFPS in phase 3 led to short-term peaks of N<sub>2</sub>O emissions with moderate intensity at days 15 (13.7 μg N<sub>2</sub>O-N kg soil<sup>-1</sup> h<sup>-1</sup>) and 29 (128.3 μg N<sub>2</sub>O-N kg soil<sup>-1</sup> h<sup>-1</sup>). Total cumulative N<sub>2</sub>O-N emissions were in the order urea > B550 > NoF > B350, being significantly higher for urea compared to the other treatments.

## 4. Discussion

### 4.1. N mineralization from biochar

N in biochar is mostly contained in aromatic moieties (Pels et al., 1995), predominantly forming pyridine (6 member-N heterocycles), pyrrole (5 member N-heterocycles), and/or pyridone structures (Knicker et al., 1996; Taherymoosavi et al., 2016). These N heterocyclic compounds are naturally present in soil and are typically assumed to be



**Fig. 4.** Cumulative CO<sub>2</sub>-C (a) and N<sub>2</sub>O-N (b) fluxes measured during the incubation. **WS:** soil amended with wheat straw; **B350:** soil amended with biochar produced from WS by slow pyrolysis at the highest heating temperature (HHT) of 350 °C; **B550:** soil amended with biochar produced from WS by slow pyrolysis at HHT of 550 °C. **UR:** Soil fertilized with urea only; **Control:** not fertilized soil. See Table 3 for the description of the three phases. Letters show significant differences (after 42 days) according to the LSD Sidak test (*P* < 0.05).

**Table 6**Cumulative CO<sub>2</sub>-C fluxes measured for each phase of the incubation and expressed as (a) mg C kg<sup>-1</sup> soil and (b) % of SOC + added C.

| (a)                        | phase 1 |   | phase 2 |   | phase 3 |    | (b)                      | phase 1 |     | phase 2 |     | phase 3 |  |
|----------------------------|---------|---|---------|---|---------|----|--------------------------|---------|-----|---------|-----|---------|--|
|                            | day 14  |   | day 28  |   | day 42  |    |                          | day 14  |     | day 28  |     | day 42  |  |
| mg C kg <sup>-1</sup> soil |         |   |         |   |         |    | % of supplied C plus SOC |         |     |         |     |         |  |
| B550                       | 195     | b | 190     | b | 518     | c  | 0.8                      | c       | 0.8 | c       | 2.1 | c       |  |
| B350                       | 268     | b | 200     | b | 520     | c  | 1.2                      | b       | 0.9 | c       | 2.3 | c       |  |
| WS                         | 1959    | a | 893     | a | 1200    | a  | 9.8                      | a       | 4.5 | a       | 6.0 | a       |  |
| UR                         | *       |   | 196     | b | 701     | b  | *                        |         | 1.4 | b       | 5.1 | b       |  |
| NoF                        | 207     | b | 197     | b | 601     | bc | 1.5                      | b       | 1.4 | b       | 4.3 | b       |  |

WS: wheat straw; B350: biochar produced from WS at the highest heating temperature (HHT) of 350 °C; B550: biochar produced from WS at HHT of 550 °C; NoF: not fertilized soil See Table 3 for the description of the three phases. Letters show significant differences between treatments according to the LSD Sidak test ( $P < 0.05$ ). \*Urea was added in the second phase of the incubation.

highly resistant to hydrolysis and microbial degradation (Schulten and Schnitzer, 1997).

Not many studies have directly looked at the mineralization of slow pyrolysis biochar-N in soil. A common proxy is the study of mineralization of pyrogenic organic matter (PyOM) resulting from wildfires. For example, de la Rosa and Knicker (2011) incubated <sup>15</sup>N-enriched PyOM from perennial ryegrass (*Lolium perenne* L.) during 72 days and concluded that 10% of the <sup>15</sup>N added to the soil was incorporated into new biomass, which demonstrated a higher bioavailability of N in PyOM than previously thought. However, the extrapolation of these results to slow-pyrolysis biochars must be made with caution (Santín et al., 2017), since the PyOM has been usually subjected to mild charring conditions compared to slow-pyrolysis biochars (e.g. 4 min at 350 °C under excess air in the above referred study). Short exposure to low temperature under oxic conditions are known to influence biochar mineralization and priming (Zimmerman et al., 2011; Fang et al., 2015; Budai et al., 2016) as well as biochar N availability (Wang et al., 2012). For example, one of the few experiments analyzing the bioavailability of slow pyrolysis biochar-N (400 °C, 30 min) produced from narrow leaf plantain (*Plantago lanceolata* L.) found a much lower proportion of <sup>15</sup>N incorporation into biomass: only 0.3% of new biomass-N was derived from the amended biochar (Jeffery et al., 2017). This result is in line with our findings, since the proportion of biochar-derived mineral N with respect to total mineral N in our soil was always lower than 0.6% and 1.6% for B550 and B350, respectively (Table 4c).

Temperatures of pyrolysis and retention times have been found to influence biochar N availability (Wang et al., 2012). It is known that with increasing thermal treatment, pyrrolic-N forms transform into pyridinic structures, with simultaneous condensation of the C matrix and N incorporation in the graphene layers (Pels et al., 1995), which progressively limits N accessibility to soil microorganisms. Therefore, the significantly lower proportion of biochar-derived inorganic N in B550 compared to B350 in our experiment is not surprising.

As hypothesized, the addition of urea in phase 2 favored the mineralization of B350-N, but not of B550-N. Although there are no studies analyzing the impact of N fertilizers on biochar-N mineralization, some studies looked at the impact of N addition on biochar C mineralization by <sup>13</sup>C and <sup>14</sup>C analysis. For instance, Jiang et al. (2016) found that the addition of NH<sub>4</sub>NO<sub>3</sub> in two N-limited soils triggered the C mineralization of a slow pyrolysis biochar (produced at 450 °C for 4 h). Between 7 and 11% extra CO<sub>2</sub> (depending on the soil) was derived from biochar when extra N was added. On the contrary, Maestrini et al. (2014) found that the addition of NH<sub>4</sub>NO<sub>3</sub> did not affect the mineralization of a similar biochar (produced under the same pyrolysis conditions).

Our results suggest that addition of available N may favor the mineralization of low-temperature biochars, but this effect might be restricted for high-temperature biochars. To our knowledge there are no isotope studies analyzing the effect of N addition on the mineralization of slow-pyrolysis biochars produced at high temperatures (550 °C or

higher).

#### 4.2. Biochar priming effect on soil native N

To date, no studies have looked at soil N priming by biochar addition, i.e. the effect that the addition of biochar to soil may have on the mineralization of N already present in soil (native N), or on N recently added with fertilizers. In our study, B550 consistently increased native NH<sub>4</sub><sup>+</sup> concentrations in all the three phases of the incubation (Fig. 2). However, this increase was not accompanied by an increase in net CO<sub>2</sub> emissions (Fig. 4), which suggests apparent positive priming, meaning that unlabeled N could have been removed from the inorganic N pool and substituted by labeled N. The main acknowledged causes of pool substitution reactions can be identified in (i) microbial immobilization and (ii) denitrification (Jenkinson et al., 1985). However, these circumstances were hardly reached in the first phase of our experiment. B550 additions did not lead to net N immobilization (Fig. S1) and, although denitrification might have contributed to a moderate extent at the beginning of phases 2 and 3, it was very unlikely in phase 1 (aerobic conditions, no N<sub>2</sub>O emissions). Therefore, the positive apparent priming effect found in this experiment is more likely a consequence of an isotopic displacement of N from a “bound” pool (i.e. SOM-N, microbial biomass-N or fixed NH<sub>4</sub><sup>+</sup>). The two first pools are very unlikely to have contributed since: (i) SON is covalently bound in complex molecules and it does not exchange with NO<sub>3</sub><sup>-</sup> or NH<sub>4</sub><sup>+</sup> (Knicker, 2011), (ii) exchange between exocellular NH<sub>4</sub><sup>+</sup> and endocellular NH<sub>4</sub><sup>+</sup> (or N in amide groups) in the microbial biomass is, in general, very unlikely to contribute in an appreciable way (Jenkinson et al., 1985). However, biochar might have contributed to an increased NH<sub>3</sub> retention capacity due to its adsorption properties and porous structure, modifying in this way the sorbed NH<sub>3</sub>. The ability of biochar to retain NH<sub>4</sub><sup>+</sup> has been demonstrated in batch adsorption experiments (Gai et al., 2014; Wang et al., 2016a). Moreover, biochar has been found to sorb NH<sub>3</sub> in moderately acidic soils in field experiments, being available to plants (Taghizadeh-Toosi et al., 2012a, b).

#### 4.3. Combined effect of biochar and glucose-C addition on inorganic N dynamics in soils

One of the most interesting findings of our study is the interaction of biochar with glucose and its effect in both native and urea derived N concentrations in phase 3. Glucose addition prompted an increase in CO<sub>2</sub> fluxes in all treatments, along with a net N immobilization. However, both biochars significantly reduced CO<sub>2</sub> emissions compared to the urea treatment (Table 6) and counteracted the immobilization of NH<sub>4</sub><sup>+</sup> caused by the increased microbial activity (Figs. 2 and 3). Our results suggest that biochar may partially offset the mineralization of easily available organic C, buffering the immobilization of inorganic N in soil when fresh organic inputs are incorporated.

Several studies have reported a negative priming effect on the



mineralization of the most readily mineralizable SOC fractions when biochar was co-applied with easily mineralizable carbon sources (Whitman et al., 2014b). For instance, Whitman et al. (2014a); Keith et al. (2015) showed that biochar amendment partially offset the positive rhizosphere priming effect or reduce it further where it was already negative. They attributed this effect to the sorption of plant root exudates onto the biochar surface thus limiting the availability of dissolved organic C for microbial use. Similarly, Lu et al. (2014) found that biochar decreased dissolved organic C in soil and exerted a negative priming effect on SOM mineralization and related this phenomenon to adsorption of dissolved organic C. In an experiment with  $^{14}\text{C}$ -labeled glucose Quilliam et al. (2013) observed a lower microbial mineralization of glucose on the biochar external and internal surfaces than in the control bulk soil and the soil immediately surrounding the biochar, which could also be related to protection mechanisms.

The sorption of organic compounds to biochar surface has been reported not only in soil, but also in composting settings with much higher organic C loads (Sanchez-Monedero et al., 2018). However, as pointed out by Pignatello et al. (2017) sorption is a complex function of biochar characteristics and solute molecular structure, and not easily predictable.

In a recent study Farrell et al. (2015) analyzed the effect of biochar on low molecular weight carbon (LMW-C) cycling in soil and established that it depends on both the biochar and the soil properties, although overall they concluded that high-temperature biochars tend to slow the microbial utilization of LMW-C. This could have important implications not only for the C cycle in soil, but also for N availability as we have demonstrated here.

#### 4.4. Implications of biochar use in agricultural ecosystems

Our study gives a new perspective on using biochar amendment to modulate N availability to crops under different N fertility management: First, the positive priming effect observed in biochar amended soils implies higher availability of native mineral N to plants, allowing the reduction of external N inputs to crops. The fact that it is an “apparent priming effect” indicates that the mineral N is not a consequence of higher SON mineralization, but of higher retention of the SON mineralized in soil, in particular  $\text{NH}_4^+$ . Second, the short term increase of urea derived  $\text{NH}_4^+$ -N in biochar amended soils, indicates that biochar can be used to raise-up fertilizer use efficiency in fast growing crops characterized by high N requirements within a very short period after their emergence (e.g. leafy vegetables as rocket, lettuce). Third, biochar amendment reduced the N immobilization due to external C inputs, which allows the design of fertilization strategies aimed at favoring soil C build-up through a combined use of biochar and other organic amendments (e.g. compost), without risk of reducing fertilizers N efficiency. In addition the low availability of N added with biochar supports the hypothesis of its very slow mineralization, allowing an increase in the soil carbon stock (Huang et al., 2018). Finally, our results confirm the ability of biochar to reduce C fluxes from agricultural soils, supporting the key role of this material for increasing soil carbon storage (Brassard et al., 2018; Huang et al., 2018). We expect our results to be applicable to other biochars produced from straws (barley, wheat, oat, etc.) under similar pyrolysis conditions.

#### 5. Conclusions

We found that wheat straw biochars produced at 350 and 550 °C and applied at a rate of 1.5% did not immobilize inorganic N in soil. Moreover, significantly higher native  $\text{NH}_4^+$  concentrations were found throughout the incubation when B550 was added, which we attribute to an apparent priming effect due to an increased capacity of biochar amended soil to retain  $\text{NH}_4^+$ . On the other hand, biochar addition did not modify native  $\text{NO}_3^-$  concentrations in soil. Biochar interacted with urea by increasing the ratio of  $\text{NH}_4^+/\text{NO}_3^-$  of urea-derived inorganic

N. When glucose was added, biochar offset the  $\text{NH}_4^+$ -N immobilization caused by glucose addition in soil. In this case, there was a significant net decrease in  $\text{CO}_2$  emissions from soils amended with biochar, which points to a limiting effect of biochar on glucose mineralization. This effect might have important implications for soil C and N management, since the reduced degradation of easily mineralizable C inputs led to a lower immobilization of both native and urea-derived  $\text{NH}_4^+$  in soil. Our results point to the importance of coupled C-N mechanisms underlying the priming effect, as well as the need of distinguishing between “real” and “apparent” priming.

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#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.soilbio.2019.01.005>.

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