Nitrogen mineralisation from spent mushroom substrates during their short-term incubation with calcareous soils

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Abstract
The aim of this work was to study the effect of the addition of spent mushroom substrates on the nitrogen mineralisation dynamics of two soils, with different texture. Related to this aim, an incubation controlled experiment was carried out during 105 days, using two calcareous soils with different textures (clay-loam and sandy-loam, respectively). Four treatments were applied: control without amendment (C), spent mushroom substrate from *Agaricus bisporus* crop (SMS-AB), spent mushroom substrate from *Pleurotus* crop (SMS-P) and a mixture of spent mushroom substrates from *Agaricus bisporus* crop and from *Pleurotus* crop (50% v/v) (SMS-50). N_{org}, NH_4^+-N and NO_3^--N concentrations and N losses were measured in the soil at 0, 3, 6, 10, 14, 21, 35, 49, 70 and 105 days of incubation. The incorporation of the wastes into the soils produced an increase in the contents of N_{org} compared to those observed in the control treatment, especially in the case of SMS-AB treatment. In both soil types, in general, the NH_4^+-N concentration values in the amended soils were statistically similar to those of the control throughout the experiment. However, the nitrate production was higher in the unamended soils in comparison to the soils with wastes. The pesticides applied to the substrates during mushroom production probably inhibited the nitrification process in the soil. Regarding soil type, the N immobilisation was higher in the most fine-textured soils (S1), since in this type of soil was observed the lowest cumulative mineralised N. The N_{org} losses were higher in the sandy-loam soils than in the clay-loam soils, this parameter being higher in the soils amended with SMS-AB and SMS-50.

Introduction

Spent mushroom substrates (SMS) are generated from the edible mushroom industry, one of the most important agro-based industries in Spain, which produces 131,000 tonnes/year of mushrooms [1]. This industry produces two main types of waste substrates, one for *Agaricus bisporus* (SMS-AB) and other for *Pleurotus* (SMS-P). SMS-AB is composed of a composted mixture of cereal straw and manure (poultry and/or horse manure and/or pig slurry), calcium sulphate, soil and residues of inorganic nutrients and pesticides, whereas SMS-P contains fermented cereal straw and residues of inorganic nutrients and pesticides [2]. For the production of each kilogram of mushroom, 5 kg of these waste substrates are produced [3]. So, approximately 655,000 tonnes of waste substrates are generated per annum from different Spanish mushroom industries. As an alternative to their disposal as waste, the spent mushroom substrates can be used in soil and water bioremediation [4, 5], in pest control of crops [6, 7], and as livestock feed [8], energy feedstock [3], growing media [7] and organic amendment [9]. However, only the agricultural use is an economically and ecologically acceptable way to dispose of them. Therefore, the aim of the present work was to study the effect of SMS and the type of the used calcareous soil on the nitrogen mineralisation in the amended soils.

Material and Methods

Four treatments, in a completely-randomised design with three replicates per treatment at each sampling date, were used in a short-term incubation experiment. The treatments were: control without amendment (C); SMS-AB, SMS-P and a mixture of SMS-AB and SMS-P at 50% (w/w) (SMS-50). The main characteristics of these wastes were: pH 5.97, 8.71 dS m^{-1} electrical conductivity (EC), 71.1 % organic matter (OM), 2.46 % N_{org}, 93 mg kg^{-1} NH_4^+-N and 66 mg kg^{-1} NO_3^--N for SMS-AB; pH 5.08, 4.03 dS m^{-1} EC, 89.1 % OM, 1.23 % N_{org}, 28 mg kg^{-1} NH_4^+-N and 19 mg kg^{-1} NO_3^--N for SMS-P and pH 5.52, 6.37 dS m^{-1} EC, 78.3 % OM, 1.85 % N_{org}, 61 mg kg^{-1} NH_4^+-N and 43 mg kg^{-1} NO_3^--N for SMS-50.The spent mushroom substrates were incorporated into two different textured soils (S1 and

S2), using an application rate of 25 g waste (dry weight) kg^{-1}. The soils used were S1, a calcareous clay-loam Xerofluvent [10] collected from Orihuela (Alicante, Spain) and S2, a calcareous sandy-loam Haplocalcid [10] from Guardamar del Segura (Alicante, Spain). The main characteristics of the soils were: 13.6 % active $CaCO_3$, pH 8.35, 0.44 dS m^{-1} EC, 8.76 g kg^{-1} organic carbon (C_{org}), 1399 mg kg^{-1} N_{org}, 14 mg kg^{-1} NO_3^--N and 1.5 mg kg^{-1} NH_4^+-N for S1 and 9.9 % active $CaCO_3$, pH 8.71, 0.17 dS m^{-1} EC, 5.39 g kg^{-1} C_{org}, 872 mg kg^{-1} N_{org}, 3.4 mg kg^{-1} NO_3^--N and 1.2 mg kg^{-1} NH_4^+-N for S2. N_{org}, NH_4^+-N and NO_3^--N concentrations and N losses were studied in destructive samples at 0, 3, 6, 10, 14, 21, 35, 49, 70 and 105 days of incubation.
Soil texture and active $CaCO_3$ contents in the soil samples, OM in the waste substrates and pH, EC, C_{org}, N_{org}, NO_3^--N and NH_4^+-N in both soil and compost samples were determined according to the methods described by Bustamante et al. [11]. N_{org} loss was calculated according to the equation described by Hernández et al. [12]. All analyses were made in triplicate. Confidence intervals at $P < 0.05$ were calculated for all figures to compare the effect of the different treatments.

Results and discussion

The addition of the spent mushroom substrates produced, in general, an increase of the N_{org} concentration throughout the incubation experiment in comparison to the control soils, this increase being higher in the SMS-AB soils (Figures 1a and 1b). Also, the increase of the N_{org} concentration was higher in the clay-loam soils (S1), due to this soil already showed higher basal N_{org} contents than S2. The highest increases of the organic N concentration in the amended soils coincided with the periods in which lost N content was negative (N gain) (Figures 1g and 1h). This fact could be due to N immobilisation processes [13] or due to the nitrogen biological fixation. This nitrogen biological fixation could be related to the increase of the bacterial community in the soil, such as free-living N_2-fixing bacteria, with the application of spent mushroom substrates [14].
On the other hand, the NH_4^+-N concentration values in the amended soils were statistically similar to those of the control soils, except in the case of SMS-AB and SMS-50 treatments in S2 (Figures 1c and 1d). This fact may be related to gaseous NH_3-losses, since nitrification process was inhibited in this experiment, as it will be commented later on. In general, the transformation of NH_4^+-N into NO_3^--N was reduced in the amended soils, this nitrification inhibition being lower in the SMS-AB soils (Figures 1e and 1f). In both soils types, the final NO_3^--N contents in the amended soils were similar. However, the nitrification inhibition was higher in S1 than in S2. This nitrification inhibition was also observed by Stewart et al. [15], when spent mushroom substrate was used as an organic amendment. The inhibition of the nitrification could be due to the pesticides applied to the substrates during mushroom production [16]. Stewart et al. [15] observed that the formaldehyde application, as a sterilant of substrate following cropping, caused a decrease in the cumulative amount of inorganic-N leached from mushroom-substrate-amended soil.
The evolution of the N losses showed that the amended soils had low N losses, observing a N gain during the first days and central period of the incubation experiment, whereas in the unamended soil, the lost N content was noticeable (Figures 1g and 1h). This behaviour suggests that mineralisation processes predominated in the control soil, N being lost by NH_3 or N_2O volatilisation. However, in the amended soils, the stimulation of the microbial development and activity induced by the added spent mushroom substrate meant that N fixation and immobilisation processes predominated over mineralisation processes, the losses of N being less noticeable, especially in the case of SMS-P treatment. Also, the N_{org} losses were higher in the sandy-loam soils than in the clay-loam soils.
It is important to remark the low N losses observed from the amended soil, aspect essential from the point of view of the contribution of agriculture to greenhouse gas emissions. Agricultural land is the most important source of N_2O emissions, contributing approximately 46–52% of the global anthropogenic N_2O flux [17]. N_2O is produced during numerous nitrogen transformations in soils, but on most occasions denitrification and nitrification are the main sources [18].

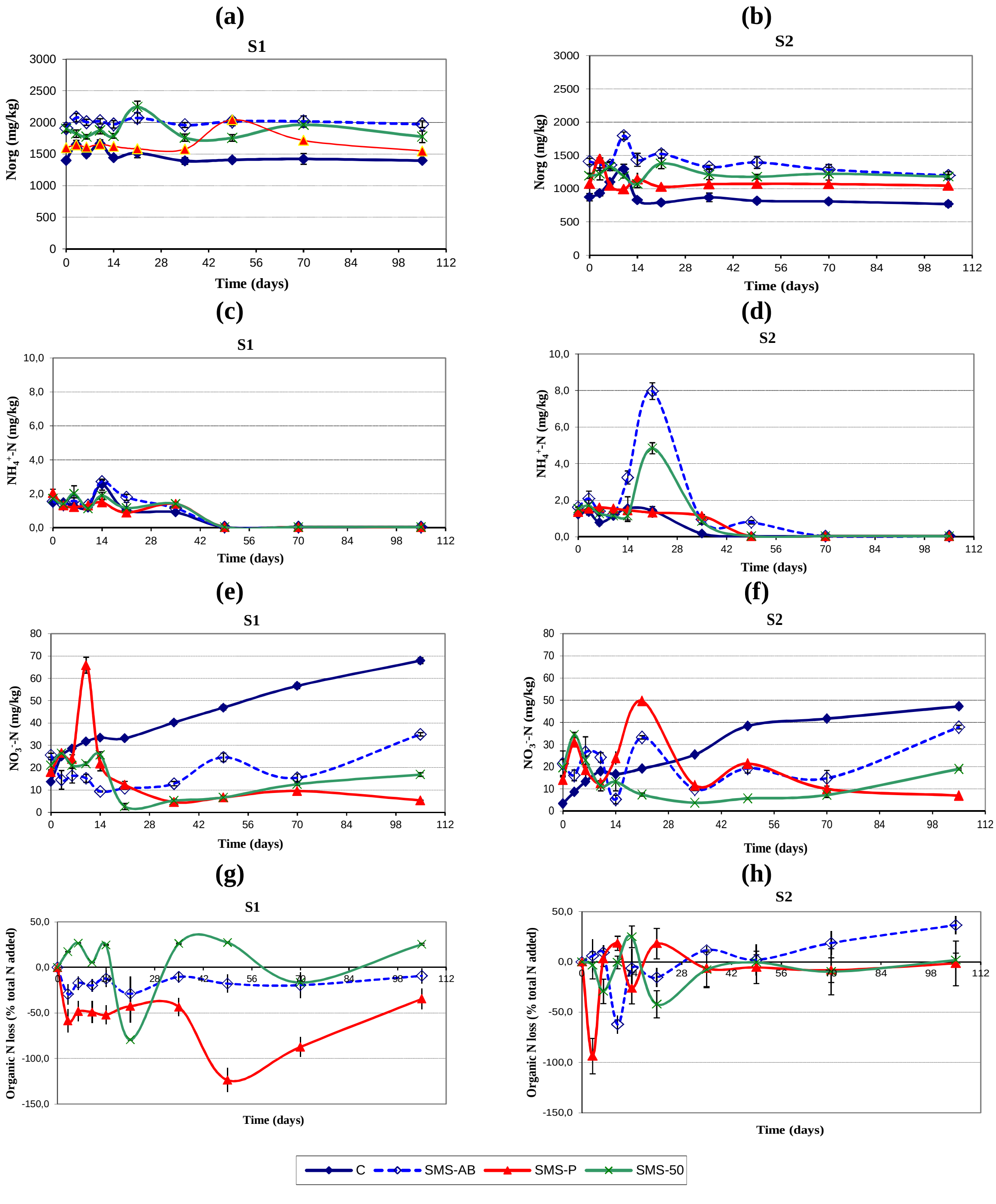

Figure 1 Evolution of N_{org} (a and b); NH_4^+-N (c and d) and NO_3^--N (e and f) contents and waste N_{org} losses (g and h) in amended and control soils S1 (clay-loam soil) a) and S2 (sandy-loam soil) during the incubation experiment (C: control; SMS-AB: spent mushroom substrate from *Agaricus bisporus* crop; SMS-P: spent mushroom substrate from *Pleurotus* crop and SMS-50: a mixture of spent mushroom substrates from SMS-AB and SMS-P (50% v/v)).

Conclusions and perspectives

The soil application of spent mushroom substrates improved soil fertility, since soil organic N was increased significantly by the organic fertilisation with these substrates, this soil fertility improvement being higher with SMS-AB treatment. However, low N mineralisation of these materials, and even a period of N immobilisation, was observed, especially in SMS-P soils, this fact contributing to low N losses. These low N losses from SMS amended soils constitute an essential aspect from the point of view of the contribution of agriculture to greenhouse gas emissions. The type of soil principally influenced the nitrification process and N losses.

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