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ORIGINAL PAPER

Immobilization and mineralization of nitrogen in a saline and alkaline soil during microbial use of sugarcane filter cake amended with glucose

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Abstract A 42-day incubation was conducted to study the effect of glucose and ammonium addition adjusted to a C/N ratio of 12.5 on sugarcane filter cake decomposition and on the release of inorganic N from microbial residues formed initially. The CO_2 evolved increased in comparison with the non-amended control from 35% of the added C with pure +5 mg g^{-1} soil filter cake amendment to 41% with +5 mg g^{-1} soil filter cake +2.5 mg g^{-1} soil glucose amendment to 48% with 5 mg g^{-1} soil filter cake +5 mg g^{-1} soil glucose amendment. The different amendments increased microbial biomass C and microbial biomass N within 6 h and such an increase persisted. The fungal cell-membrane component ergosterol initially showed a disproportionate increase in relation to microbial biomass C, which completely disappeared by the end of the incubation. The cellulase activity showed a 5-fold increase after filter cake addition, which was not further increased by the additional glucose amendment. The cellulase activity showed an exponential decline to values around 4% of the initial value in all treatments. The amount of inorganic N immobilized from day 0 to day 14 increased with increasing amount of C added, in contrast to the control treatment. After day 14, the immobilized N was re-mineralized at rates between 1.3 and 1.5 μg N g^{-1} soil d^{-1} in the treatments being more than twice as high as in the control treatment. This means that the re-mineralization rate is independent of the actual size of the microbial residues pool and also independent of the size of the soil microbial biomass.

Keywords Microbial biomass C · Microbial biomass N · Ergosterol · CO_2 production · N re-mineralization

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Introduction

In countries with a sugarcane-based sugar industry, such as Pakistan or India, sugarcane filter cake ("pressmud") has the potential to be an important C source for subtropical soils poor in organic matter (Badole et al. 2001; Yadvinder-Singh et al. 2008). However, the addition of sugarcane filter cake to moderately acidic and also to alkaline soils led to immobilization of inorganic N (Rasul et al. 2006; Khan et al. 2008). This immobilization is rather surprising as the ratios of soil organic C to total N and 0.5 M K_2SO_4 extractable organic C to organic N in the sugarcane filter cake were below 15, where no N immobilization should occur (Powlson et al. 2001). The decomposition of an easily available carbohydrate fraction, mobilized for example during drying, seems to be responsible for the rapid immobilization directly after the amendment of sugarcane filter cake to soil (Khan et al. 2008). During later stages of the incubation, inorganic N was slowly released from the decomposition of the microbial residues (Khan et al. 2008).

The addition of an easily available C source such as glucose certainly increases the initial immobilization of inorganic N (Chander and Joergensen 2001; Dilly and Nannipieri 2001). However, the release of inorganic N might be disproportionately increased later, due to priming effects, which has also been observed after combined amendments of glucose and ammonium to saline and alkaline soils (Luna-Guido and Dendooven 2001; Conde et al. 2005). Mimicking a labile component of root exudates, for example, glucose amendments have also been shown to trigger microbial activity for certain periods (De Nobili et al. 2001) and to increase litter decomposition (Kuzyakov et al. 2007). For this reason, an incubation study was conducted to answer the questions whether combined glucose and ammonium amendments adjusted to a C/N ratio of 12.5 affect (1) filter cake decomposition and (2) the release of inorganic N from microbial residues formed initially.

Materials and methods

Soil and amendments

A bulk sample of 20 kg arable soil was taken at 0–15 cm depth from the Gujranwala district, Punjab province (33.36°N and 73.07°E) of Pakistan during July 2002 (Muhammad et al. 2006). The soil was classified as Solonchak according to the FAO-WRB (world reference base) classification system and had a pH in water of 8.23±0.04 (SD=standard deviation), a $CaCO_3$ content of 16.1% ±0.2, a salt content of 6.0±1.0 mg g^{-1} soil with an electrical conductivity of 16.0±2.0 mS cm^{-1}, and a sodium absorption ratio (SAR) of 8.5±0.2. The texture of the soil was 25.2% ±0.7 sand, 64.7% ±1.1 silt, and 10.1% ±0.5 clay, containing 5.4±0.6 mg organic C, 0.8±0.1 mg total N, and 0.67±0.09 mg total P g^{-1} soil (Muhammad et al. 2006). Inorganic N extractable with 0.5 M K_2SO_4 was initially 87.8±0.8 µg NO_3^-N g^{-1} soil and 0.2±0.1 µg NH_4^+–N g^{-1} soil, when the experiment started. The sugarcane filter press cake ("pressmud") was taken from an industrial dump of the Hussain Sugar Mill, Jaranwalda, in the District of Faisalabad, Pakistan, air dried, homogenized, and sent to Germany. The sugarcane filter cake contained the following element and nutrient concentrations g^{-1} dry weight (±SD): 440±8.0 mg C, 34±1.4 mg total N, 12.3±0.3 mg total P, 3.9±0.1 mg total S, 11.7±0.3 mg K, 3.9±0.1 mg Mg, and 23.4±0.5 mg Ca.

Incubation procedure

The soil was adjusted to 40% of its water holding capacity, homogenized, and pre-incubated at room temperature for 2 weeks prior to treatment application. The treatments included: (1) non-amended control, (2) +5 mg g^{-1} soil sugarcane filter cake, (3) +5 mg g^{-1} soil sugarcane filter cake+2.5 mg g^{-1} soil glucose+8 mg $(NH_4)_2SO_4$ g^{-1} soil, and (4) +5 mg g^{-1} soil sugarcane filter cake+5 mg g^{-1} soil glucose+16 mg $(NH_4)_2SO_4$ g^{-1} soil. The organic amendments of treatments (2), (3), and (4) were equivalent to 2,000, 3,000, and 4,000 µg C g^{-1} soil adjusted to a C/N ratio of always 12.5. For each treatment, 200 g (oven-dry basis) soil were placed in quadruplicate into 3-l incubation vessels. The moisture was adjusted with demineralized water or amendment solution to 50% water holding capacity. Then, the incubation vessel was tightly closed and incubated for 42 days at 30°C in the dark. The CO_2 evolved was absorbed in 1 M NaOH solution and analyzed by back titration with 1 M HCl. After 2, 4, 8, 14, 21, 28, 35, and 42 days of incubation, the incubation bottles were opened and aerated during the change of the 1 M NaOH solution. Soil (50 g oven-dry weight) was collected at 0, 14, 28, and 42 days after incubation and analyzed for different microbial biomass and activity indices.

Microbial biomass and K_2SO_4-extractable components

Microbial biomass C and microbial biomass N were estimated by fumigation–extraction (Brookes et al. 1985; Vance et al. 1987). Two portions of 10-g moist soil were taken from the 50-g soil sample. One portion was fumigated for 24 h at 25°C with ethanol-free $CHCl_3$. Following fumigant removal, the soil was extracted with 40 ml 0.5 M K_2SO_4 and filtered. The non-fumigated portion was extracted similarly at the time fumigation commenced. Organic C in the extracts was measured as CO_2 by infrared absorption after combustion at 850°C using a Dimatoc 100 automatic analyzer (Dimatec, Essen, Germany). Microbial biomass C was calculated as E_C/k_{EC}, where E_C is (organic C extracted from fumigated soils) minus (organic C extracted from non-fumigated soils) and k_{EC} is 0.45 (Wu et al. 1990). Total N in the extracts was measured by chemoluminescence detection after combustion (Dima-N, Dimatec). Microbial biomass N was calculated as E_N/k_{EN}, where E_N is (total N extracted from fumigated soils) minus (total N extracted from non-fumigated soils) and k_{EN} is 0.54 (Brookes et al. 1985; Joergensen and Mueller 1996). In the 0.5 M K_2SO_4 extracts of non-fumigated soil samples, NO_3^-N and NH_4^+–N were additionally measured using segmented continuous flow analysis followed by spectrometric detection. Extractable organic N was calculated as the difference between extractable total and inorganic N.

Ergosterol and cellulase activity

Ergosterol was measured in 0.5 g filter cake and in 2 g of moist soil taken from incubation vessels after 0 and 42 days

of incubation. The soil samples were extracted with 100 ml ethanol by oscillating shaking for 30 min at 250 rpm (Djajakirana et al. 1996). Ergosterol was determined by reversed-phase HPLC with 100% methanol as the mobile phase and an absorbance of 282 nm. Cellulase activity in the soil samples taken from incubation vessels after 0, 14, 28, and 42 days of incubation was determined as described by Alef and Nannipieri (1995). One gram of moist soil was incubated with 5 ml acetate buffer and 0.5 g avicel for 16 h at 40°C. After centrifugation (2,500 ×*g*, 10 min), reducing sugars in the supernatants were estimated as reported by Nelson and Somogyi (Spiro 1966).

Statistical analysis

The results presented in the tables are arithmetic means of four replicates and expressed on an oven-dry basis (about 24 h at 105°C). The significance of treatment effects on K_2SO_4 extractable C, ergosterol, and the ergosterol-to-microbial biomass C ratio was tested by a one-way analysis of variance for the sampling days 0 and 42 using the Scheffé post-hoc test. All statistical analyses were performed using Stat View 5.0 (SAS Inst. Inc.).

Results

The cumulative amount of CO_2 evolved during the 42-day incubation increased with increasing amount of carbon (Table 1). However, not only the absolute amount increased but also the relative percentage, from 37% of the added C with pure filter cake amendment (+5 mgF) to 41% with +5 mg g^{-1} soil filter cake+2.5 mg g^{-1} soil glucose amendment (+5 mgF+2.5 mgG) to 48% with +5 mg g^{-1} soil filter cake+5 mg g^{-1} soil glucose amendment (+5 mgF+5 mgG).

The content of K_2SO_4-extractable C decreased in the control treatment by 35% during the 42-day incubation (Table 1). After the organic amendments, the content of K_2SO_4 extractable C declined to values around 42 µg g^{-1} soil, which were identical to the initial content. The amount of glucose extractable at day 0, i.e. 6 h after addition, was equivalent to 75% of the added glucose C in the treatments +5 mgF+2.5 mgG and +5 mgF+5 mgG. Also, the amount of extractable inorganic N was equivalent to 75% of the added NH_4^+ at day 0 in these two treatments (Fig. 1). The amount of inorganic N immobilized from day 0 to day 14 increased with increasing amount of C added in comparison to the control from 27 µg N g^{-1} soil (+5 mgF) to 82 µg N g^{-1} soil (+5 mgF+2.5 mgG) and to 135 µg N g^{-1} soil (+5 mgF+5 mgG). The amounts of N additionally immobilized by glucose addition were 55 and 108 µg N g^{-1} soil, corresponding to 69% and 68% of the NH_4^+–N added initially. After day 14, the immobilized N was constantly re-mineralized at a rate of 1.3 µg N g^{-1} soil day^{-1} in the sole filter cake treatment and at a rate of 1.5 µg N g^{-1} soil day^{-1} in the two glucose treatments. In the control treatment, the N mineralization rate was constantly at 0.64 µg N g^{-1} soil day^{-1} throughout the experiment. Less than 0.5% of inorganic N was extracted as NH_4^+, i.e., more than 99.5% were extracted as NO_3^- since day 14 of the incubation (results not shown).

Microbial biomass C remained constant at 127 µg g^{-1} soil throughout the incubation in the control treatment (Fig. 2a). Microbial biomass N followed with larger variations (Fig. 2b) at a roughly constant microbial biomass C-to-N ratio of 12 (Fig. 2c). With pure filter cake amendment, the microbial biomass C content increased from 184 µg g^{-1} soil at day 0 to 328 µg g^{-1} soil at day 28, followed by a decline that did not return to the initial value (Fig. 2a). In the two glucose treatments, the maximum microbial biomass C contents were measured at day 0, exceeding four and six times the microbial biomass C of the control soil. Then, the microbial biomass C content declined until the end of the experiment, reaching contents

Table 1 Cumulative CO_2 evolution over the 42-day incubation at 30°C; contents of 0.5 M K_2SO_4-extractable C and ergosterol and the ratio of ergosterol-to-microbial biomass at the beginning and at the end of the incubation experiment

Treatment	ΣCO₂–C (µg g^{-1} soil)	0.5 M K_2SO_4-extractable C (µg g^{-1} soil)		Ergosterol (µg g^{-1} soil)		Ergosterol/microbial biomass C (%)	
		Day 0	Day 42	Day 0	Day 42	Day 0	Day 42
Control	387 d	43 d	28 b	0.15 d	0.11 d	0.12 b	0.10 a
+5 mgF	1,120 c	134 c	40 a	0.51 c	0.18 c	0.28 a	0.08 b
+5 mgF +2.5 mgG	1,630 b	879 b	40 a	0.76 b	0.23 b	0.15 b	0.09 ab
+5 mgF+5 mgG	2,320 a	1,624 a	45 a	1.17 a	0.25 a	0.15 b	0.08 b
CV (±%)	1	2	6	7	5	8	8

F Sugarcane filter cake amendment in mg g^{-1} soil; *G* glucose amendment in mg g^{-1} soil; *CV* mean coefficient of variation between replicate measurements. Different letters indicate a significant difference ($P<0.05$, Scheffé, $n=4$)

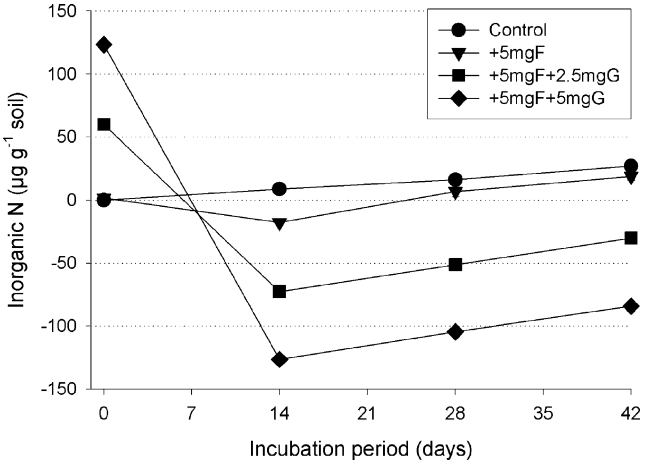

Fig. 1 Changes in the content of 0.5 M K_2SO_4-extractable inorganic N ($NO_3^-N+NH_4^+–N$) during the 42-day incubation at 30°C. *F* Sugarcane filter cake amendment in mg g^{-1} soil; *G* glucose amendment in mg g^{-1} soil; one mean standard deviation was ±2.6 µg g^{-1} soil, which is smaller than the symbols in all cases. The mean coefficient of variation is 20% ranging from 2% to 130%

below 50% of the maximum content. With pure filter cake amendment, microbial biomass N content followed that of the control on a 4 µg g^{-1} soil or 40% larger level throughout the incubation (Fig. 2b). In the treatment +5 mgF+ 2.5 mgG, microbial biomass N was constant at 22 µg g^{-1} soil throughout the incubation, but in treatment +5 mgF+ 5 mgG microbial biomass N showed a 23% decline down to 28 µg g^{-1} soil at the end of the incubation. At this time, the microbial biomass C/N ratio of the two glucose treatments was identical to that of the control treatment, which was always significantly exceeded by that of the pure filter cake amendment (Fig. 2c).

The organic amendments always led to an immediately apparent 3- to 8-fold increase in ergosterol within 6 h after addition, compared with the control soil (Table 1). However, the filter cake contained 115±12 SD ergosterol g^{-1} dry matter. This means that already 0.58 µg ergosterol g^{-1} soil were added with the amendments. This amount of ergosterol would be equivalent to 62% (+5 mgF), 105%

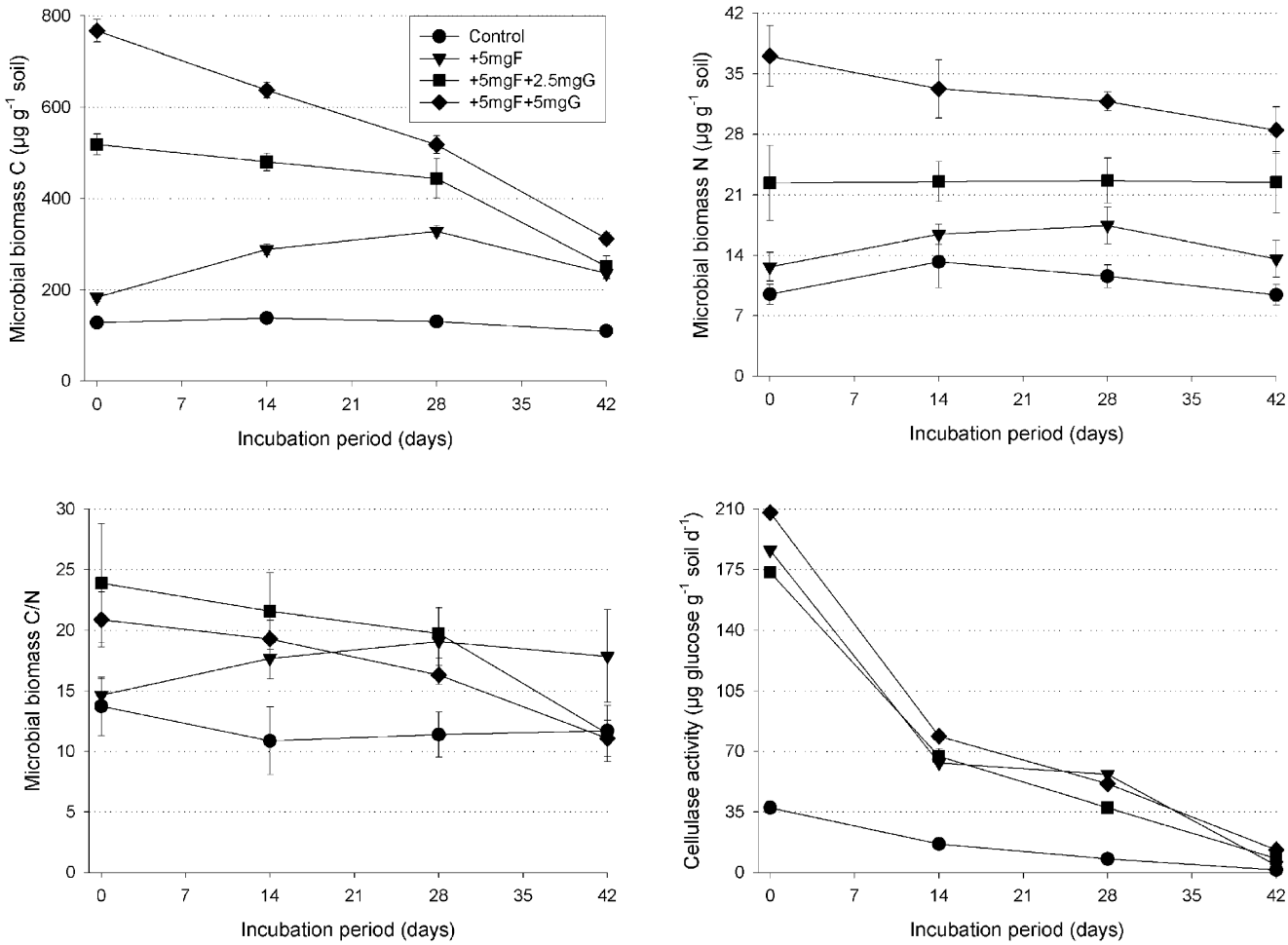

Fig. 2 Changes in **a** the contents of microbial biomass C, **b** the contents of microbial biomass N, **c** the microbial biomass C/N ratio, and **d** cellulase activity during the 42-day incubation at 30°C; *F* Sugarcane filter cake amendment in mg g^{-1} soil; *G* glucose amendment in mg g^{-1} soil. *Bars* indicate ±1 standard deviation, but they are not always larger than the symbols

(+5 mgF+2.5 mgG), and 176% (+5 mgF+5 mgG) of the amount measured at day 0 after application of the amendments and subtracting the ergosterol content of the control soil. The increase in ergosterol was stronger than that of microbial biomass C, leading to an increased ergosterol-to-microbial biomass C ratio with the pure filter cake amendment. In the control, the ergosterol-to-microbial biomass C ratio declined only slightly until the end of the incubation. In contrast, those ratios with the amendments were always below that of the control at the end of the incubation, in most cases significantly. The addition of sugarcane filter cake led to an immediate 5-fold increase in cellulase activity, which was only slightly increased by the additional glucose amendment (Fig. 2d). The cellulase activity showed an exponential decline to values around 4% of the initial value in all treatments. However, with glucose addition, cellulase activity was slightly higher at the end of the incubation.

Discussion

Microbial activity

Stimulation of CO_2 production in the present highly saline and moderately alkaline soil in response to the organic amendments indicated the capability of soil microorganisms to survive and perform their metabolic functions under salt stress conditions (Luna-Guido and Dendooven 2001; Conde et al. 2005). The actual percentage of organic C mineralized in the sugarcane filter cake amended soil was similar to that reported for acidic (Rasul et al. 2006) and for alkaline soils (Khan et al. 2008) at different degrees of salinity. Assuming that glucose addition did not affect the decomposition of autochthonous soil organic matter and that of sugarcane filter cake, 51% of the added glucose was decomposed in the treatment with 0.25% glucose and 60% in the treatment with 0.5% glucose amendment. This relative increase in CO_2 production with increasing addition rate of glucose indicates a priming effect (Kuzyakov et al. 2000) repeatedly observed after glucose amendments in incubation experiments (Falchini et al. 2003; Hamer et al. 2004). The glucose addition probably increased both the decomposition rate of autochthonous soil organic matter and that of sugarcane filter cake.

Cellulase activity was immediately increased by sugarcane filter cake amendment, indicating the presence of both cellulose and cellulases in this material. A close relationship between the presence of cellulose and cellulase activity has been observed repeatedly (Linkins et al. 1990; Kautz et al. 2004). Pavel et al. (2004) detected highest cellulase activity between 7 and 14 days after the amendment of cellulose or plant material, respectively. The strong decline by 95% of cellulase activity during the incubation suggests the depletion of cellulose in the remaining sugarcane filter cake material. A less strong decrease in cellulase activity was found by Parthasarathi and Ranganathan (2000) during a 30-day aging period of sugarcane filter cake vermicasts. However, the cellulase activity showed also a 95% decline in the non-amended control soil, whereas the CO_2 production rate declined by 20% from the 0–2 days' period to the 35–42 days' period (results not shown). This suggests that cellulases are rapidly inactivated after depletion of the substrate and that cellulase activity makes only a minor contribution to the basal respiration of a soil.

Retardation or complete inhibition of nitrification at high salt concentrations was observed by Darrah et al. (1987) in acidic soils and Pathak and Rao (1998) in highly alkaline soils, artificially salinized. Oren (1999) stated that the energy burden of saline environments might be too great for the ammonia or nitrite-oxidizing autotrophic microorganisms. Complete inhibition of nitrification was also reported by Rasul et al. (2006) in a saline acidic German soil. However, this was not observed in the saline and moderate alkaline soil used in the present study in accordance with Luna-Guido et al. (2000), studying alkaline soils adapted for long periods to saline conditions. In such adapted soils, salinity effects on C and N mineralization, but obviously also nitrification were regularly smaller than in artificially salinized soils (Khan et al. 2008).

During the first 14 days of incubation, the organic amendments resulted in a rapid immobilization of N, despite a low C/N ratio of 12.5. Ammonium losses did not occur in accordance with Pathak and Rao (1998), investigating soils at similar or even higher pH. The relationship between glucose and NH_4^+ indicates a biological reason for such microbial immobilization. The amount of N immobilized was considerably higher as compared to the corresponding increases in soil microbial biomass N, suggesting that large amounts of inorganic N were immediately transferred into the fraction of microbial residues (Vinten et al. 2002; Mayer et al. 2004). From days 14 to 42, the N re-mineralization rate from these freshly formed microbial residues was similar with pure filter cake amendment to that in the two glucose treatments and more than doubled in comparison with that of the older autochthonous soil organic matter. The parallel increase in re-mineralization rate with all three amendments was in clear contrast to the disproportionate increase in the CO_2 production rate with increasing glucose amendment rates. This suggests that priming effects mainly affected exclusively N-free soil organic matter or sugarcane filter cake fractions.

Microbial biomass

The sole addition of sugarcane filter cake led to an immediate increase in microbial biomass C and N, as observed by Rasul et al. (2006), contrasting the results of Dee et al. (2003). It is likely that this processed material is colonized during drying in open pits by microorganisms that recover immediately during rewetting, as observed by De Nobili et al. (2006) or Formowitz et al. (2007) after a certain period of storage under air-dried conditions. The average C/N ratio of the microbial biomass was 18 with the amendments and thus considerably exceeded the C/N ratio of the amendments. This indicates that factors other than N limitation must cause this feature typical of many tropical soils (Formowitz et al. 2007; Muhammad et al. 2008). However, strongly increased microbial biomass C/N ratios have also been observed in a German soil after glucose addition (Chander and Joergensen 2007).

The immediate uptake of glucose and NH_4^+ into the microbial biomass within the first few hours after amendment is another important feature of the present results. Roughly 25% of the glucose and 25% of the NH_4^+ added remained non-extractable with 0.5 M K_2SO_4 in soil within 6 h, regardless of the portion added. Similar rapid glucose uptake rates by soil microorganisms have been observed by Coody et al. (1986), Anderson and Gray (1990), and Jones and Murphy (2007). This percentage must be taken up by soil microorganisms. The net increases in microbial biomass C accounted for 130% (+5 mgF+2.5 mgG) and 115% (5 mgF+5 mgG) of the loss in glucose C after 6 h, respectively. In contrast, the net increase in microbial biomass N accounted for only 50% (+5 mgF+2.5 mgG) and 66% (+5 mgF+5 mgG) of the loss in NH_4–N. These percentages are based on the use of the average k_{EC} and k_{EN} values for dormant, i.e., non-growing microbial populations. These conversion values are not necessarily true for microorganisms after glucose addition. Joergensen and Raubuch (2002) observed an uptake of glucose without further metabolism of the taken up glucose. This non-metabolized glucose is of course fully extractable after fumigation. This decoupling of glucose uptake and respiration has recently been confirmed by Hill et al. (2008). It should be noted that the initial glucose and NH_4^+–N uptake was solely dependent on the concentrations added and was completely independent of the ratio of substrate to microbial biomass.

Some filter cake-colonizing fungi, which recovered immediately by rehydration during rewetting (Formowitz et al. 2007), seem to react metabolically very fast on easily available substrate. This is suggested by the increase in ergosterol content in the two glucose treatments during the 6 h after amendment. These fast-growing fungi might be yeasts that contain very high ergosterol concentrations in comparison with other fungal species (Arnezeder and Hampel 1990; Pasanen et al. 1999). The decrease in the ergosterol-to-microbial biomass C ratio indicates a shift in the fungal community structure towards autochthonous fungi, which exhibit very low ergosterol-to-microbial biomass C ratios, as reported by Rasul et al. (2006). The shift in the fungal community structure towards autochthonous fungi is in line with the transient increase in microbial biomass C by glucose addition, which declined to the level with the pure filter cake amendment at the end of the incubation. The constancy of microbial biomass after glucose addition seems to confirm the hypothesis of a constant biological space in each soil with a stable microbial biomass, whose level is supported by native available organic C (Nannipieri et al. 1983). In contrast, the addition of sugarcane filter cake seems to add new microbial microsites for survival in soil as reported for ryegrass in comparison with glucose (Wu et al. 1993). The low percentage of NH_4^+–N, which may account for the net increase in microbial biomass N at day 0, indicates that a certain amount of microbial biomass N must be rapidly transferred into the fraction of microbial residues. However, the present experiment cannot explain whether these residues are derived from freshly formed biomass or by replacing older, soil organic matter-derived biomass.

Conclusions

The addition of sugarcane filter cake adds new microbial microsites for survival in soil, in contrast to glucose. The relative increase in CO_2 production with increasing addition rate of glucose indicates a priming effect due to the decomposition of more recalcitrant factions. The addition of sugarcane filter cake and glucose in combination with NH_4 led to an immediate immobilization of inorganic N in microbial residues. The re-mineralization rate of these freshly formed microbial residues is more than twice as high as that of the older autochthonous soil organic matter. It is a striking feature of the present experiment that the N re-mineralization rate was similar with all three amendments from days 14 to 42. This means that the re-mineralization rate is independent of the actual size of the microbial residues pool and also independent of the size of the soil microbial biomass. Other unknown soil properties seem to form a soil-specific gate for the release of inorganic N. Despite the strong re-mineralization rate, the N immobilization effect of sugarcane filter cake should be considered if used as fertilizer.

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