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Salinity-induced changes in the microbial use of sugarcane filter cake added to soil

Dissertation

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by

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dedicated to

My Parents

Muhammad Siddiq and Noori Begum

My loving wife Razia Sultana

and children

Falaq Rasul, Muhammad Afzal, Fatima Rasul and Buraq Rasul

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Chapter 1 - General introduction

1.1 Agriculture in Pakistan

Pakistan is an agro-based country of the sub-continent situated between the Himalayan mountains and the Arabian Sea at longitude 61° and 76° E and latitude 24° and 37° N. The climate of Pakistan is sub-tropical to semi-arid, having annual rainfall ranges from 125 mm in the extreme southern plains to 500 to 900 mm in the sub-mountainous and northern plains. Except in the mountainous areas, summer is very hot, with a maximum temperature exceeding 45°C, while the minimum in winter is a few degrees above freezing point. Pakistan consists of four provinces, Punjab, Sindh, NWFP and Balochistan, plus federally administered Tribal Areas (Figure 1), with a total geographical area of 796,096 km^2 (Punjab 205,344; Sindh 140,914; NWFP 74,521; Balochistan 347,190, FATA 27,220 and Islamabad Federal Area 906), corresponding to 20.6 million ha, 14.1 million ha, 10.2 million ha, 34.5 million ha for the four provinces of Pakistan, respectively. The cultivable area of Pakistan is 35.4 million ha, forestland accounts for 3.5 million ha, cultivable waste 8.6 million ha, cultivated area 22 million ha and the salt affected area is 6.3 million ha. Most of the areas in the Punjab and Sindh provinces are comprised of plain land, formed by the River Indus. Pakistan is known for its excellent canal irrigation system and rich agricultural lands. Presently, Pakistan is in the grip of a massive population explosion and has experienced greater population growth than other developing countries. The current population of Pakistan is above 145 million and it is increasing at 2.61% per annum, increasing the gap between the supply of and demand for agricultural products (Alam and Naqvi, 2003). To meet the challenge to food supply posed by the burgeoning population of Pakistan, there is an urgent need to boost crop yield.

In Pakistan, agriculture is the largest income-generating sector, accounting for about 35 to 40% of the national income and employing more than 45% of the country's total labor force, and supports directly or indirectly about 68% of the population for their sustenance. It contributes about 65% to total export earnings derived from raw and processed agricultural commodities. It provides food, feed and raw materials for major industries, such as textiles, sugar and to several other medium and small-scale industries, which account for about 50% of the total value of industrial production. It is thus evident that the welfare of the vast majority of the population is critically

dependent upon efficient utilization of the agricultural resources of the country on a sustainable basis.

Fig. 1: Map of Pakistan

The crop yield in Pakistan is lower than that of agriculturally advanced countries. The general problems associated with agriculture of this region are scarcity of water, floods, water logging, salinity and alkalinity, soil erosion, low yield per unit area, and traditional methods of cultivation. The most fundamental constraint in Pakistan is water availability, which limits further expansion of agriculture, so its efficient use must be given high priority. Other general problems that contribute to the low yield and poor quality of crops include poor quality seeds, poor soil management, low yielding varieties, lack of crop protection methods, shortage of irrigation water, credit facilities and non-application of modern technology in raising crops. Thus, Pakistan faces a major challenge to improve crop productivity per unit of land to ensure national food security in the wake of growing population. Most of the soils are poor in organic matter, normally below 1%. This poor status of organic matter in soils and non-availability of other nutrients restrict the sustainable agriculture in Pakistan.

1.2 Salinization of agricultural lands

Salinization is the process of accumulation of salts in the surface soil layers (Muhammad, 1996). A soil is considered saline where the electrical conductivity of a saturated paste extract (ECe) exceeds 4 dS m^{-1} (Northcote and Skene, 1972). At this ECe, the composition and concentration of salts in the soil solution adversely affects plant growth by limiting the absorption of water (osmotic effects) and through specific ion effects, which can induce ion toxicities (Keren, 2000). Sodication refers to the accumulation of exchangeable sodium on the surface of soil clay minerals (Muhammad, 1996). Soil sodicity is expressed in terms of Exchangeable Sodium Percentage (ESP), and a soil possessing ESP greater than 15 is known to be sodic in nature. Sodicity results in numerous adverse phenomena in soil, such as dispersion and unstable structure due to high exchangeable Na^+ and pH, restricted permeability to air and water, nutrient imbalances, including Ca^{2+}, K^+, Cu, Zn, Fe, Mn, and specific ion toxicity, like Na^+, Mo and Mg^2.

Salinization is one of the major problems responsible for decline in soil productivity in many countries of the world (Keren, 2000; Rietz and Haynes, 2003). It is worth mentioning that no agriculturally important continent and climate zone is free of salt-affected soils, though the extent varies. The increasing frequency of dry periods in several regions of the world frequently results in the occurrence of salinity on cultivated

lands (Hu and Schmidhalter, 2005). Currently, about 50% of the world's irrigated lands, which are at least twice as productive as rain-fed lands and produce up to one-third of the world's food, are affected by salinization (Ghassemi et al., 1995; Hillel, 2000).

Climate is a key factor in the economics and sustainability of soils and their productive utilization. Salinization is a characteristic of arid and semi-arid environments, especially where surface irrigation is practiced. In Pakistan (longitude 61º and 70º E and latitude 24º and 37º N), a vast tract of land (6.3 million ha) out of 80 million hectares of total geographical area has become salt-affected (Muhammad, 1996), particularly due to such agro-climatic conditions. Many farmers consider salinity to be "land cancer" which affects 30% of arable land and is a big threat to crop production (Qureshi and Barret-Lenard, 1998). The main causes of salinity include seepage from canals, high salt concentration in irrigation water, insufficient leaching of salts and use of irrigation water with a poor salt balance. The salt-affected soils are mainly confined to the Indus Plains and extend over almost the entire irrigated area, apart from a few localized places. About 56% of the salt-affected soils of Pakistan and 80% of those of the Punjab province are saline-sodic. The magnitude of the salinity and sodicity problem can be gauged from the fact that at one stage about 40,000 ha a^{-1} were becoming salt-affected in Pakistan. The productivity of these soils is extremely low, which makes their cultivation unprofitable.

In view of the growing menace of salinity and sodicity in Pakistan and other countries of the world, a large number of studies have been conducted in order to evaluate the impact of salts in soil plant systems. The main focus of such studies, however, has been the negative effect of salts on soil physico-chemical properties, while the microbiological properties of soils have largely been neglected. In Pakistan particularly, the negative effects of salts on physical and chemical properties of soils are well documented (Qureshi and Barret-Lenard, 1998), but studies regarding impacts of salts on soil microbiological properties, particularly on the size and activity of soil microbial biomass, are clearly lacking.

1.3 Use of organic substances in saline agriculture

Saline agriculture is an alternative approach to soil reclamation for obtaining better crop production from salt-affected soils (Ghafoor et al., 2004). This technique aims at improving the physico-chemical properties of salt-affected soils through the use of

different organic resources, in order to keep the land economically productive. Incorporation of organic substrates is of prime importance to maintain soil fertility, productivity and soil organic matter (SOM) and counteract nutrient depletion in salt-affected soils of tropical regions like Pakistan. These soils are highly depleted, due to intensive cropping and raising more and more crops over the year to fulfill the food requirement of the growing population. Incorporation of organic manures in agricultural systems is an important factor in the control of soil fertility and maintenance of soil organic matter. If such measures are not taken in time, then soil depletion and deterioration of fertility and productivity go hand in hand. In this study, the main thrust is to seek and explore new organic resources for amelioration, as well as increasing the crop production in saline and alkaline areas of Pakistan.

1.4 Sugarcane filter cake

The sugar industry produces a number of by-products during the process of sugar production including bagasse, mill mud or filter cake, ash, mill effluent, and trash. Sugarcane filter cake is the solid material left after filtering cane juice. These by-products may be used as a source of energy but also as a source of nutrients and as soil ameliorates. The re-use of mill by-products has been mutually beneficial to the farming community and the milling sectors as well as supporting the industry's endeavors to be viewed as clean, green and responsible (Barry et al., 2000). Sugar mills generate between 20 and 60 kg of mud per ton of cane crushed (Chapman, 1996). Harvesting conditions affect the extraneous matter and soil content of the cane supply, which, in turn, affects the quantity of mud. In Pakistan, there are presently 71 sugar mills generating 2 million t a^{-1} filter cake, which average about 28,170 t per mill a^{-1}. Punjab province has 39 sugar mills and in most of the sugarcane producing districts there is more than one mill.

Heavy addition of mill mud altered the texture of the soil, turning it from hard setting to soil that was loose and friable. By increasing the availability of water to the crop, the mill mud reduced the effects of salinity on crop growth (BSES, 1994). In crop production, imbalances, under dosing and unsubsidized prices are acute problems associated with fertilizers. Erratic supply of imported fertilizers, of phosphorus and potash is often experienced at sowing time of main crops. Soils are severely deficient in organic matter. On average, they contain 0.1-0.2% SOM, which is far below the

satisfactory starting level of 0.86. Due to low humus status, water-holding capacity, soil structure, and colloidal exchange complex are not very conducive to achieving optimum crop production. Generally, being saline-alkaline in nature and having a pH normally above 8.5 and a high salt level, the soil restricts favorable crop yields. Luckily, sugarcane filter cake, a compound waste product of the sugar cane industry has a considerable quantity of organic matter, macro and micronutrients and soil amendment characteristics, due to sulfur it contains.

1.5 Dhancha (*Sesbania bispinosa*)

Sesbania bispinosa (Jacq.), also known as *Sesbania aculeata*, is a member of the *Fabaceae* family. Locally it is known as Dhancha. It is an erect annual or biennial legume, which reaches a height of about 3.5 meters. In crowded stands it grows tall and straight. Its leaves are narrow and oblong. The plant consists of a raceme bearing between 3 and 12 flowers on short pedicles. The pods are slender and contain 35-40 seeds. The seeds are dark brown and are about 4 millimeters long. *Sesbania bispinosa* is commonly grown for the reclamation of salt-affected soils. It is adapted to a variety of soil conditions, varying from waterlogged to saline, and from sands to clays. It has a 50% decrease in growth in soils with an ECe of 13 dSm^{-1} (Qureshi and Barret-Lenard, 1998).

Sesbania bispinosa is native to Pakistan and India. It is commonly grown in the plains and foothills of all four provinces of Pakistan. It is drought resistant and grows from March to September/October. It grows up to an altitude of 1200 meters and within a rainfall range of 550 to 1100 mm. It grows vigorously after the plants have become established and reaches a height of about 2 meters in 3 months. It is one of the best available green manuring crops and is widely used in reclamation of salt-affects soils. It fixes large amounts of nitrogen; a good crop can add 5000-7000 kg of organic matter and 85-110 kg of N per hectare. The root system greatly helps in opening up the soil and acidifying the root environment through the production of carbon dioxide and acidic exudates. It is high quality fodder, rich in protein (18%) and minerals (9%). Animals relish it because of its succulence and large foliage. The sticks of dhancha are extensively used for roofing mud houses and as fuel wood.

1.6 References

Alam, S.M., Naqvi, M.H., 2003. Pakistan agriculture - 2003, status paper "The gap between the supply and demand of agricultural product". Tando Jam, Pakistan. May, pp. 19-25.

Barry, G.A., Rayment, G.E., Bloesch, P.M., Price, A., 2000. Recycling sugar industry by-products and municipal biosolids on canelands. In: Bruce, R.C, Johnstone, M., Rayment, G.E. (Eds.), Environmental Short Course for Sustainable Sugar production: Course Manual. Working Paper, Cooperative Research Centre for Sustainable Sugar Production, Townsville.

Batra, L., Manna, M.C., 1997. Hydrogenase activity and microbial biomass carbon in salt-affected soils of semi arid and arid regions. Arid Soil Research and Rehabilitation 11, 295-303.

BSES,1994. Salting effects reduced by mill mud. BSES Bulletin, No. 46, April, pp. 10-11.

Chander, K., Brookes, P.C., Harding, S.A., 1995. Microbial biomass dynamics following addition of metal-enriched sewage sludges to a sandy loam. Soil Biology & Biochemistry 27, 1409-1421.

Chapman, L.S., 1996. Australian sugar industry by-products recycle plant nutrients. In: Hunter, H.M., Eyles, A.G. Rayment, G.E. (Eds.), Downstream Effects of Land Use. Department of Natural Resources, Brisbane.

Dar, G. H., 1996. Effects of cadmium and sewage sludge on soil microbial biomass and enzyme activities. Bioresource Technology 56, 141-145.

Federal Bureau of Statistics, 2004. Statistical Year Book. Ministry of Finance, Government of Pakistan, pp.83-98.

Flowers, T.J., Yeo, A.R., 1995. Breeding for salinity resistance in crops plants: Where next? Australian Journal of Plant Physiology 22, 875-884.

Ghafoor, A., Qadir, M., Murtaza, G., 2004. Salt-affected Soils, Principles of Management. Allied Book Center, Lahore, Pakistan.

Ghassemi, F., Jakeman, A.J., Nix, H.A., 1995. Salinisation of Land and Water Resources. University of New South Wales Press, Canberra, Australia.

He, Z. L., Yao, H.Y., Chen, G.C., Zhu, J., Huang, C.Y., 1997. Relationship of crop yield to microbial biomass in highly weathered soils of China. In: Ando, T., Fujita, K., Mae, T., Matsumoto, H., Mori, S., Sekiya, J. (Eds.), Plant Nutrition for Sustainable Food Production and Environment. Kluwer, Dordrecht, pp.751-752.

Hu, Y., Schmidhalter, U., 2005. Drought and salinity: A comparison of their affects on mineral nutrition of plants. Journal of Plant Nutrition and Soil Science 168, 541-549.

Jamil, A., Iqbal, M., Yaqub, M., 1990. Chemical composition of filter press-cake of different sugar mills in Faisalabad division. Pakistan Sugar Journal IV(1), 27-32.

Jenkinson, D.S., Ladd, J.N., 1981. Microbial biomass in soil: measurements and turnover. In: Paul, E.A., Ladd, J.N. (Eds.), Soil Biochemistry, Volume 5. Dekker, New York, pp. 415-471.

Keren, R. 2000. Salinity. In: Summer, M. E. (Ed.), Handbook of Soil Science, CRC, Press, Boca Raton, pp. 3-25.

Khan, K.S., 2003. Dynamics of microbial biomass C, N and P in rainfed soils under wheat and maize production. Research Report Pakistan Science Foundation, Islamabad.

Lee, K.E., Pankhurst, C.E., 1992. Soil organisms and sustainable productivity. Australian Journal of Soil Research 30, 855-892.

McGrath, S.P., Chaudhri, A.M., Giller, K.E., 1995. Long-term effects of metals in sewage sludge on soils, microorganisms, and plants. Journal of Industrial Microbiology 14, 94-104.

Muhammad, S., 1996. Soil salinity, sodicity and water logging. In: Rashid A., Memon, K.S., (Eds.), Soil Science. National Book Foundation, Islamabad, pp.471-508.

Nelson, P.N., Ladd, J.N., Oades, J.M., 1996. Decomposition of ^{14}C-labelled plant material in salt-affected soil. Soil Biology & Biochemistry 28, 433-441.

Northcote K.H., Skene J.K., 1972. Australian soils with saline and sodic properties. CSIRO, Soil Publication No. 27.

Pathak, H., Rao, D.L.N., 1998. Carbon and nitrogen mineralization from added organic matter in saline and alkali soils. Soil Biology & Biochemistry 30, 695-702.

Post, W.M., Emanuel, W.R., Zinke, P.J., Stangenberger, A.G., 1982. Soil carbon pools and world life zones. Nature 298, 156-159.

Qureshi, R.H., Barrett-Lennard, E.G., 1998. Saline agriculture for irrigated land in Pakistan. Australian Centre for International Agriculture Research Canberra, Australia.

Rasul, G., Appuhn, A., Müller, T., Joergensen, R.G., 2006. Salinity induced changes in the microbial use of sugarcane filter cake added to soil. Applied Soil Ecology 31, 1-10.

Razzaq, A., 2001. Assessing sugarcane filter cake as crop nutrients and soil health ameliorant. Pakistan Sugar Journal. VI(3), 15-17

Rietz, D.N., Haynes, R.J., 2003. Effects of irrigation-induced salinity and sodicity on soil microbial activity. Soil Biology & Biochemistry 35, 845-854.

Sardinha, M., Müller, T., Schmeisky, H., Joergensen, R.G., 2003. Microbial performance in a temperate floodplain soil along a salinity gradient. Applied Soil Ecology 23, 237-244.

Sarig, S., Fliessbach, A., Steinberger, Y., 1996. Microbial biomass reflects the nitrogen and phosphorus economy of halophytes grown in salty desert soil. Biology and Fertility of Soils 21, 128-130.

Smith, J.L., Paul, E.A., 1990. The significance of soil microbial biomass estimations. In: Bollag, J.M., Stotzky, G., (Eds.), Soil Biochemistry, Volume 6. Dekker, New York, pp. 357-396.

Sparling G.P., 1992. Ratio of microbial biomass carbon to soil organic carbon as a sensitive indicator of changes in soil organic matter. Australian Journal of Soil Research 30, 195-207.

Zahran, R.R., Moharram, A.M., Mohammad, H.A., 1997. Some ecological and physiological studies on bacteria isolated from salt-affected soils of Egypt. Journal of Basic Microbiology 35, 269-275.

Chapter 2 – Salinity-induced changes in the microbial use of sugarcane filter cake added to soil

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2.1 Abstract

A laboratory experiment was carried out to prove the hypothesis that the decomposition of a complex organic substrate is reduced by the lower content of fungal biomass in a saline soil in comparison to a non-saline soil under acidic conditions. Three different rates (0.5, 1.0, and 2.0%) of sugarcane filter cake were added to both soils and incubated for 63 days at 30°C. In the saline control soil without amendment, cumulative CO_2 production was 70% greater than in the corresponding non-saline control soil, but the formation of inorganic N did not differ between these two soils. However, nitrification was inhibited in the saline soil. The increase in cumulative CO_2 production by adding filter cake was similar in both soils, corresponding to 29% of the filter cake C at all three addition rates. Also the increases in microbial biomass C and biomass N were linearly related to the amount of filter cake added, but this increase was slightly higher for both properties in the saline soil. In contrast to microbial biomass, the absolute increase in ergosterol content in the saline soil was on average only half that in the non-saline soil and it showed also strong temporal changes during the incubation: A strong initial increase after adding the filter cake was followed by a rapid decline. The addition of filter cake led to immobilisation of inorganic N in both soils. This immobilisation was not expected, because the total C-to-total N ratio of the filter cake was below 13 and the organic C-to-organic N ratio in the 0.5 M K_2SO_4 extract of this material was even lower at 9.2. The immobilisation was considerably higher in the saline soil than in the non-saline soil. The N immobilisation capacity of sugarcane filter cake should be considered when this material is applied to arable sites at high rations.

Keywords: Microbial biomass C; Biomass N; Ergosterol, CO_2 production, N mineralization

2.2 Introduction

Decomposition of organic substrates and the release of nutrient elements are key functions of soil microorganisms (Swift et al., 1979). In agricultural land, the decomposition of organic matter added by farmers, such as farmyard manure or compost, is a key component in management practices for maintaining soil fertility (Chen and Avnimelech, 1986). Important threats to the functioning of soil microbial processes are acidity (He et al., 2003) and salinity (Zahran, 1997). Both soil properties are known to have depressive effects on the soil microbial biomass (Anderson and Joergensen, 1997; Batra and Manna, 1997; Rietz and Haynes, 2003). Acidification is known to reduce generally the bacterial part of the microbial biomass (Blagodatskaya and Anderson, 1998). In contrast, salinisation reduces preferentially the fungal part of the soil microbial biomass in alkaline sandy loams in South Australia (Pankhurst et al., 2001), but also in acidic sandy loams in Germany (Sardinha et al., 2003). The specific reduction of fungi may reduce the decomposition of complex organic material in saline soils (Badran, 1994), because this group of organisms is especially important for the breakdown of lignin and cellulose in decaying plant residues (Swift et al., 1979, Harper and Lynch, 1985).

Acidity is a common feature of many soils in humid central Europe, especially of forest soils but also of extensively used grassland soils (Joergensen, 1995). In contrast, salinity is not a major threat to agricultural land in humid central Europe. However, saline soils exist, e.g. in the floodplains of different German rivers caused by potassium mining (Westhus et al., 1997; Schmeisky and Podlacha, 2000). The flora of these inland saline sites extensively used as grassland is protected by law in nature conservation areas. However, knowledge about the soil microbial processes maintaining the vegetation is very much restricted (Sardinha et al., 2003). Assessing the long-term stability of soil microbial processes is vital for further protection of these labile ecosystems. In some cases, salinity of these inland saline sites is combined with acidic soil conditions, which is a less common combination of soil properties than saline alkaline soils. However, this combination exists in subtropical India (Muralidharan and Rattan, 2001) and probably also in other countries with saline soils derived from non-carbonaceous stones and sediments. In saline and acidic soils, the decomposition of complex organic materials is presumably reduced due to the lower content of fungal biomass.

To prove this hypothesis, we designed a decomposition study in the laboratory to assess the reaction of the soil microorganisms, especially of fungi, to the addition of a complex organic substrate. Sugarcane filter cake has been proven to be preferentially decomposed by fungi (Xavier and Lonsane, 1994) and was chosen for this reason as an example of an organic amendment. This industrial organic waste material is, of course, not common in Germany, but it has an important potential as a C source in arid and semi-arid countries such as Pakistan or India (Badole et al., 2001). There, salinity is a major threat to agriculture in combination with very low levels of soil organic matter. An increased knowledge about the behaviour of this material in soil, e.g. the release of nutrients, would be helpful in these areas.

2.3 Materials and Methods

2.3.1 Soils and sugarcane filter cake

Bulk samples of 10 kg were taken at 0-5 cm depth on 24 May 2002 from two sites 80 m apart used as meadow in the floodplain of the river Werra close to Heringen in North Hessia, Germany (Schmeisky and Podlacha, 2000; Sardinha et al., 2003). At these sites, saline liquid residues originally injected into the geological underground from a potassium plant nearby have been rising to the soil surface for over fifty years. The saline site had no vegetation. The vegetation of the non-saline site was dominated by *Alopecurus pratensis*, *Poa pratensis*, *Arrhenatherum elatius*, and *Festuca rubra.* The non-saline soil can be classified as a Dystric Fluvisol and the saline soil as Salic Fluvisol according to the WRB classification system (Bailly et al., 1998). The properties of the two soils are shown in Table 1. 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, homogenised and sent to Germany. The sugarcane filter cake contained the following element and nutrient concentrations g^{-1} dry weight (standard deviation in brackets): 420 (8.0) mg C, 34 mg (1.4) 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.

All field-moist soil samples were sieved (< 2 mm), adjusted to 50% of their water holding capacity, homogenised, pre-incubated at room temperature for 2 weeks, and stored in polyethylene bags at 4°C prior to carrying out biological analyses. Soil pH was measured in 0.01 M $CaCl_2$ using a soil-to-solution ratio of 1-to-2.5. Electrical

conductivity (EC) was estimated using a soil-to-water suspension of 1-to-5, which was converted to EC values in a saturation extract (EC_e) using the following equation:

$$EC_e = EC_e \frac{WC_{1/5}}{WC_{satur}}$$

where $WC_{1/5}$ is the water content of the suspension 1-to-5 and WC_{satur} is the water content of the saturation soil paste. After removal of the salt with water, cation exchange capacity was measured according to Mehlich by elution with an unbuffered 0.1 M $BaCl_2$ solution (Schlichting et al., 1995). Subsamples of dried soil were finely ground in a ball mill. Total C and total N were determined after combustion to CO_2 and N_2 using an Elementar Vario MAX analyser (Elementar, Hanau, Germany). Concentrations of total P, total S, K, Mg, and Ca were determined by HNO_3/pressure digestion according to Heinrichs et al. (1986), as described by Chander et al. (2001) and measured by ICP-AES (Spectro Analytical Instruments/Kleve).

2.3.2 Incubation procedure

Two soils (saline and non-saline) were incubated with the following four treatments in quadruplicate: (1) control + 0% sugarcane filter cake, (2) + 0.5% sugarcane filter cake, (3) + 1.0% filter cake, and (4) + 2.0% filter cake. The addition rates were in the range used in laboratory incubation experiments (Dee et al., 2003) or field experiments (Badole et al., 2001). For each treatment, 1 kg (oven-dry basis) of soil at 50% water holding capacity plus the different amendments were weighed into 3-l incubation vessels and incubated for 63 days at 30˚C in the dark. The CO_2 evolved was absorbed in 1 M NaOH solution in 100 ml beakers. The NaOH solution was changed after 2, 4, and 7 days and thereafter weekly. Soil samples of 100 g fresh-weight were taken after 2, 4, 7, 14, 28, 42 and 63 days for the determination of microbial biomass, ergosterol, and inorganic N mineralised.

2.3.3 Microbial biomass and K_2SO_4 extractable components

Microbial biomass C and biomass N were estimated by fumigation-extraction (Brookes et al., 1985; Vance et al., 1987). Two portions equivalent to 25 g oven-dry soil were taken from the soil used for the incubation. One portion was fumigated for 24 h at 25°C with ethanol-free $CHCl_3$. Following fumigant removal, the soil was extracted with 100 ml 0.5 M K_2SO_4 by 30 min horizontal shaking at 200 rev min^{-1} 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 analyser (Dimatec, Essen, Germany). Microbial biomass C was E_C / k_{EC}, where E_C = (organic C extracted from fumigated soils) - (organic C extracted from non-fumigated soils) and k_{EC} = 0.45 (Wu et al., 1990). Total N in the extracts was measured as activated NO_2* by chemoluminescence detection (Dima-N) after combustion at 850°C. Microbial biomass N was E_N / k_{EC}, where E_N = (total N extracted from fumigated soils) - (total N extracted from non-fumigated soils) and k_{EN} = 0.54 (Brookes et al., 1985). In the 0.5 M K_2SO_4 extracts of non-fumigated soil, 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 N minus inorganic N.

2.3.4 Ergosterol

Ergosterol was measured in 4 g of moist soil taken from the soil used for the incubation. This sample was extracted with 100 ml ethanol for 30 min by oscillating shaking at 250 rev min^{-1} (Djajakirana et al., 1996). Ergosterol was determined by reversed-phase HPLC with 100% methanol as the mobile phase and a resolution of detection of 282 nm.

2.3.5 Statistics

The results presented in the tables are arithmetic means and expressed on an oven-dry basis (about 24 h at 105 °C). The significance of treatment effects on activity indices was tested either by a soil-specific one-way analysis of variance using the Tukey/Kramer HSD-test (honestly significant difference) or by a two-way ANOVA using soil and substrate (sugarcane filter cake) as independent factors. Treatment effects on microbial biomass indices were analysed by a two-way ANOVA with soil and substrate (sugarcane filter cake) as independent factors and sampling date as repeated measures. All statistical analyses were performed using StatView 5.0 (SAS Inst. Inc.).

Table 1

Properties of the non-saline and saline soil used in the incubation experiment, standard deviation in parenthesis

Properties	Non-saline soil		Saline soil	
Sand [%]	72	(0.5)	64	(4.2)
Silt [%]	12	(3.1)	30	(3.1)
Ton [%]	16	(3.4)	7	(3.6)
pH-$CaCl_2$	5.4	(0.1)	5.5	(0.1)
EC_e [mS cm^{-1}]	0.7	(0.1)	15.6	(0.6)
CEC [$\mu mol_C\ g^{-1}$]	160	(9.6)	291	(1.6)
Na [$\mu mol_C\ g^{-1}$]	8	(1.0)	124	(1.2)
K [$\mu mol_C\ g^{-1}$]	2	(0.2)	2	(0.1)
Mg [$\mu mol_C\ g^{-1}$]	14	(1.0)	64	(0.0)
Ca [$\mu mol_C\ g^{-1}$]	80	(3.0)	101	(3.6)
ESP (%)	8	(0.3)	43	(0.3)
Total C [mg g^{-1} soil]	19.2	(0.6)	38.1	(3.9)
Total N [mg g^{-1} soil]	1.8	(0.1)	3.5	(0.4)
Total P [mg g^{-1} soil]	0.42	(0.04)	1.08	(0.07)
Total C/N	10.5	(0.7)	10.8	(0.3)
Total C/P	46	(4.4) 35	(2.3)	

EC_e = electrical conductivity in a soil saturation paste, CEC = cation exchange capacity, ESP = exchangeable sodium percentage = exchangeable Na / exchangeable (Na + K + Mg + Ca) x 100

2.4 Results

In the saline control soil without amendment, cumulative CO_2 production was 70% greater than in the corresponding non-saline control after a 63-day incubation at 30˚C (Table 2). In contrast, the increase in cumulative CO_2 production by the addition of sugarcane filter cake was similar in both soils (Fig. 1), corresponding to 29% of the added C at all three addition rates. In contrast to the CO_2 production, the amount of inorganic N mineralised was identical in both control treatments, although nitrification

was completely inhibited in the saline soil (Table 2). Sugarcane filter cake had no effect on N mineralisation at the 0.5% addition rate in the non-saline soil, but led to increasing N immobilisation at the 1.0 and 2.0% addition rates. In the saline soil, significant N immobilisation occurred from the 0.5% addition rate.

In the saline control, the content of 0.5 M K_2SO_4 extractable organic C was nearly twice that in the non-saline control (Table 3). Approximately 5% of the sugarcane filter cake C was extractable by 0.5 M K_2SO_4 so that the filter cake led to a proportionate increase in line with the increasing addition rate in both soils. The fraction of 0.5 M K_2SO_4 extractable organic C showed most consistently the differences between the two soils according to the F values (Table 4). It remained roughly constant in all treatments throughout the first half of the experiment, followed by a slight decline towards the second half of the incubation period. In the saline control soil, the content of 0.5 M K_2SO_4 extractable organic N was more than twice that in the non-saline control soil (Table 3). Approximately 7% of the sugarcane filter cake N was extractable by 0.5 M K_2SO_4 as organic N so that the filter cake led to a proportionate increase in line with the increasing addition rate in both soils. Consequently, the C-to-N ratio of the extractable organic fraction decreased with increasing filter cake addition rate from 11.7 to 18.1 in the non-saline soil and from 10.7 to 15.6 in the saline soil. However, it did not show any clear trend during the incubation due to considerable variations between the different sampling dates.

The content of microbial biomass C was roughly 30% higher in the saline control than in the non-saline control (Table 4). It increased proportionately with increasing filter cake addition rate and it was always slightly higher in the saline soil. The content of microbial biomass C increased considerably in both soils within the first two days after adding the filter cake and remained roughly constant throughout the further incubation in all treatments (Fig. 2a/b). The microbial biomass N content increased also proportionately with increasing filter cake addition rate (Table 4). However, the maximum level was reached at day 7 in the non-saline soil (Fig. 3a), whereas a more continuous increase was observed in the saline soil until the end of the incubation (Fig. 3b). For this reason, the mean biomass C-to-N ratio decreased from 8.6 at day 2 to values around 6.3 from day 28 (Table 4).

Table 2

Treatment means of the cumulative CO_2-C produced, inorganic N (NH_4-N + NO_3-N), NH_4-N, and NO_3-N mineralised during a 63-day incubation period at 30ºC

	CO_2-C	Inorganic N	NH_4-N	NO_3-N
	[µg g^{-1} soil]			
Treatment means				
NS-(0%)	780 d	47.1 a	-3.3 a	50.4 a
NS-(0.5%)	1440 c	49.0 ab	-3.6 a	52.5 ab
NS-(1.0%)	2070 b	38.0 b	-3.8 a	41.8 b
NS-(2.0%)	3170 a	15.9 c	-3.5 a	19.4 c
S-(0%)	1320 d	46.3 a	45.1 a	1.2 a
S-(0.5%)	1890 c	26.6 b	26.1 b	0.5 b
S-(1.0%)	2540 b	13.4 c	12.7 c	0.7 b
S-(2.0%)	3690 a	-11.1 d	-9.6 d	-1.6 c
Analysis of variance (F values)				
Soil	195***	123***	704***	835***
Substrate	826***	130***	192***	33***
Soil x substrate	0	13***	188***	25***
CV (± %)	4	17	12	19

Negative values indicate immobilisation of inorganic N. Different letters within a column indicate a soil-specific significant difference based on a one-way ANOVA using substrate as an independent factor ($P < 0.05$; Tukey/Kramer, n = 4). F-values for a two-way ANOVA using soil and substrate (sugarcane filter cake) as independent factors; degrees of freedom: soil 1, substrate 3, soil x substrate 3. CV = mean coefficient of variation between replicate measurements (n = 4). NS = non-saline soil, S = saline soil; in brackets: added filter cake in % dry weight. *** $P < 0.001$.

Table 3
Main effects on 0.5 M K_2SO_4 extractable organic C and N, mean values of the eight treatments over the seven sampling days and mean values of seven sampling days over the eight treatments

	0.5 M K_2SO_4 extractable organic		
	C	N	C/N
	[µg g^{-1} soil]		
Treatment means			
NS-(0%)	84 d	5.4 c	18.1 a
NS-(0.5%)	104 c	7.6 b	15.3 ab
NS-(1.0%)	121 b	9.1 b	15.1 ab
NS-(2.0%)	169 a	15.1 a	11.7 b
S-(0%)	163 d	11.7 c	15.6 a
S-(0.5%)	178 c	14.2 bc	13.2 ab
S-(1.0%)	197 b	16.0 b	12.9 ab
S-(2.0%)	250 a	24.4 a	10.7 b
Day 2	160	11.7	15.3
Day 4	162	13.1	12.7
Day 7	167	12.8	13.6
Day 14	163	14.5	11.9
Day 28	158	13.8	13.1
Day 42	154	14.1	15.2
Day 63	143	10.6	16.5
Analysis of variance (F values)			
Soil	1079 ***	327 ***	15 ***
Substrate	243 ***	145 ***	22 ***
Date	11 ***	9 ***	6 ***
Soil x substrate	0.4	3.0*	0.5
Soil x date	0.7	11.5***	13.2***
Substrate x date	3.5***	3.1	3.2***
CV (± %)	7.1	17.8	17.3

Different letters within a column indicate a soil-specific significant difference based on a one-way ANOVA using substrate as an independent factor ($P < 0.05$; Tukey/Kramer, n = 28). F-values of the two-way ANOVA using soil and substrate (sugarcane filter press cake) as independent factors and sampling date as repeated measures; degrees of freedom: soil 1, substrate 3, date 6, soil x substrate 3, soil x date 6, substrate x date 18. CV = mean coefficient of variation between replicate measurements (n = 4). NS = non-saline soil, S = saline soil; in brackets: added filter cake in % dry weight; $P < 0.05$; *** $P < 0.001$.

Table 4
Main effects on microbial properties; mean values of the eight treatments over the seven sampling days and mean values of seven sampling days over the eight treatments

	Microbial biomass C ($\mu g\ g^{-1}$)	Microbial biomass N ($\mu g\ g^{-1}$)	Microbial biomass C/N	Ergosterol ($\mu g\ g^{-1}$)	Ergosterol/ microbial biomass C (%)
Treatment means					
NS-(0%)	131 d	21 d	6.8 a	1.28 c	1.04 a
NS-(0.5%)	206 c	30 c	7.1 a	1.67 bc	0.83 a
NS-(1.0%)	251 b	37 b	7.0 a	2.31 b	0.91 a
NS-(2.0%)	384 a	52 a	7.4 a	4.03 a	1.07 a
S-(0%)	169 d	27 d	6.6 a	0.43 c	0.28 a
S-(0.5%)	261 c	40 c	6.9 a	0.62 bc	0.27 a
S-(1.0%)	336 b	52 b	6.7 a	0.87 b	0.28 a
S-(2.0%)	478 a	65 a	7.4 a	1.75 a	0.40 a
Day 2	274	32	8.6	2.55	0.92
Day 4	231	29	8.1	1.85	0.74
Day 7	278	41	6.8	2.22	0.79
Day 14	279	41	6.8	1.73	0.64
Day 28	301	47	6.3	1.35	0.54
Day 42	265	44	6.2	0.85	0.40
Day 63	311	50	6.3	0.79	0.41
Analysis of variance (F values)					
Soil	101 ***	70 ***	1	258 ***	370***
Substrate	305 ***	128 ***	5 **	106 ***	6**
Date	10 ***	43 ***	26 ***	206 ***	33***
Soil x substrate	3.6*	2.0*	0.3	13.3***	1.5
Soil x date	12.8***	14.9***	1.8	25.8***	2.8*
Substrate x date	2.7***	2.9	1.5	47.8***	5.3***
CV (± %)	15.9	14.7	13.1	18.4***	27.5

Different letters within a column indicate a soil-specific significant difference based on a one-way ANOVA using substrate as an independent factor ($P < 0.05$; Tukey/Kramer, n = 28). F-values of the two-way ANOVA using soil and substrate (sugarcane filter press cake) as independent factors and sampling date as repeated measures; degrees of freedom: soil 1, substrate 3, date 6, soil x substrate 3, soil x date 6, substrate x date 18. CV = mean coefficient of variation between replicate measurements (n = 4). NS = non-saline soil, S = saline soil; in brackets: added filter cake in % dry weight; $P < 0.05$; ** $P < 0.001$, *** $P < 0.001$.

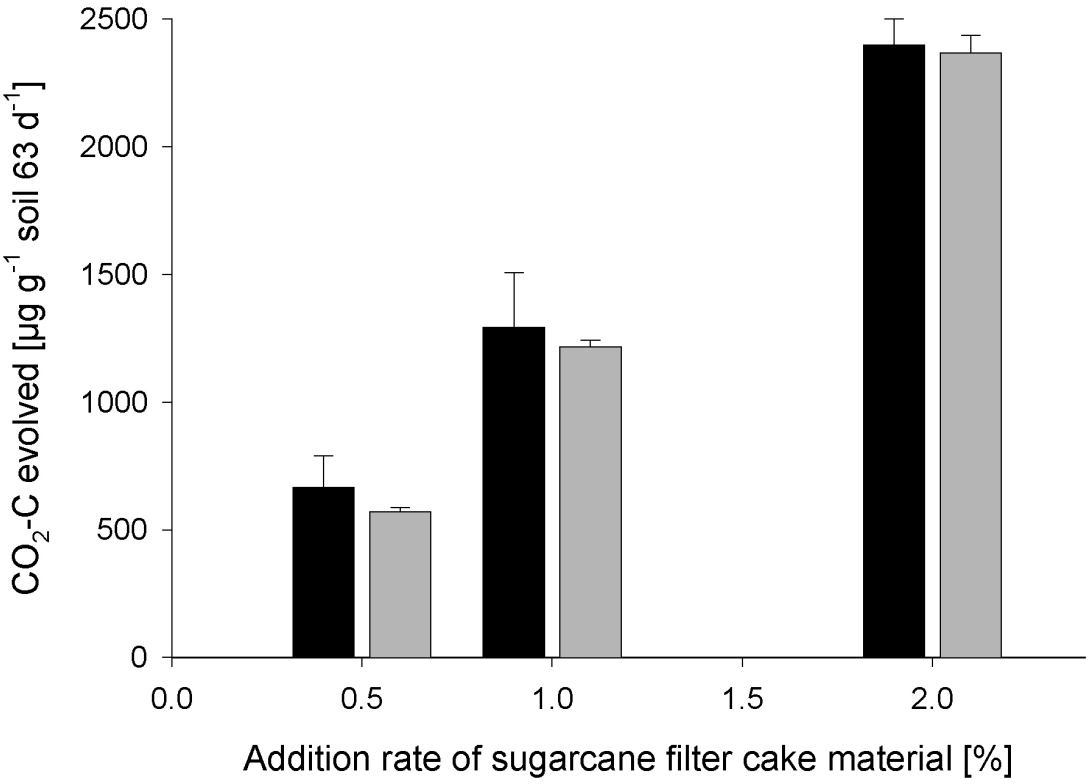

Fig. 1

Decomposition of the sugarcane filter cake during a 63-day incubation at 30°C as (CO_2-C evolved in the filter cake treatments) minus (CO_2-C evolved in the respective control treatment) in non-saline soil (▉)and saline soil (▒); bars indicate standard deviation.

The ergosterol content increased in both soils almost proportionately to the increasing filter cake addition rate. However, the ergosterol content was two to three times higher in the non-saline soil in all treatments. In contrast to microbial biomass C and biomass N, the ergosterol content (Fig. 4a/b) and consequently also the ergosterol-to-microbial biomass C ratio decreased significantly during the incubation period in both soils (Table 4). The ergosterol content revealed the strongest sampling date effects and also the strongest soil x date and substrate x date second order interactions of all microbial properties according to the F values.

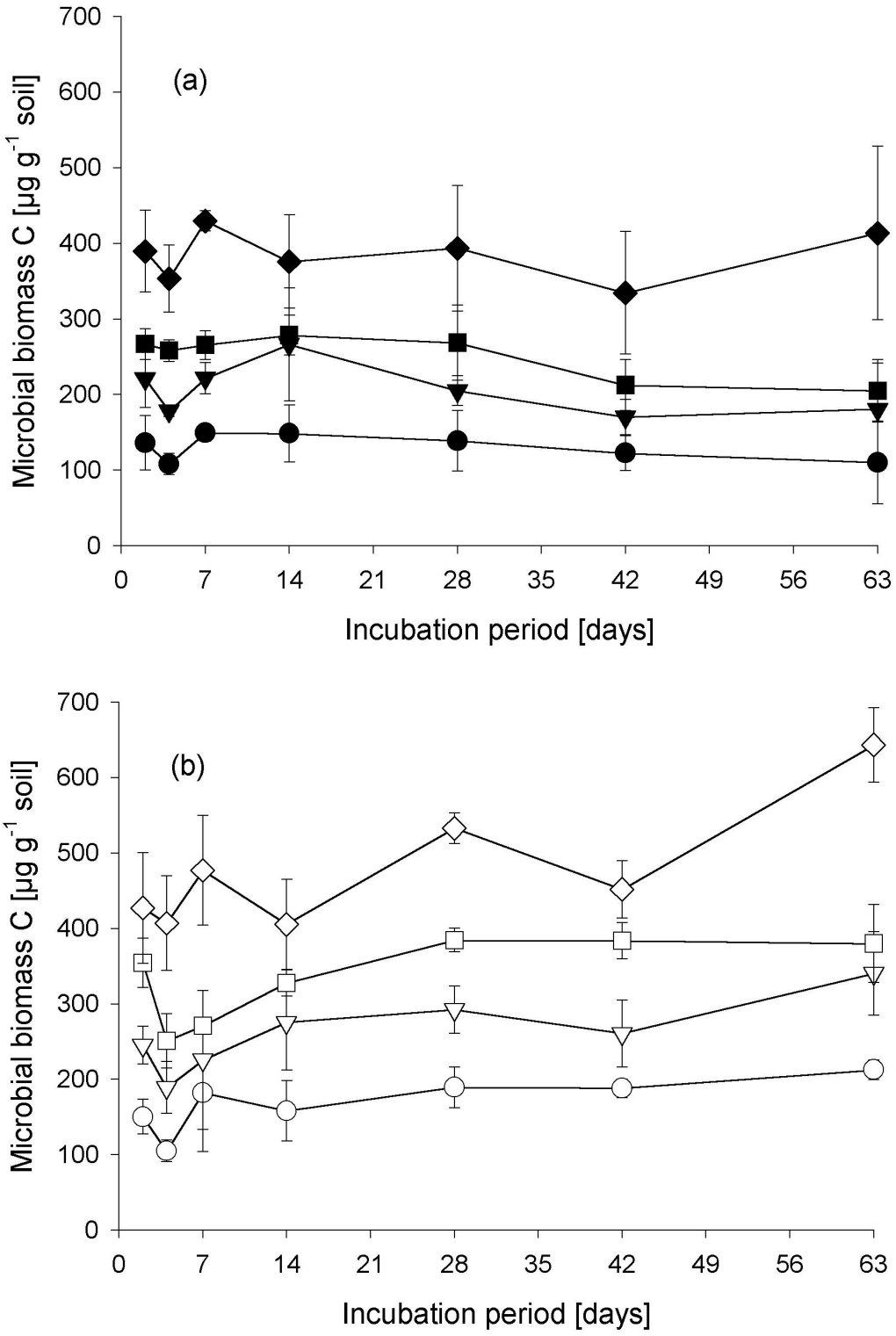

Fig. 2

Microbial biomass C content in the treatments without and with different addition rates of sugarcane filter cake (0.5, 1.0, and 2.0%) at 7 sampling dates during a 63-day incubation at 30°C; (a) non-saline soil: 0% (▼), 0.5% (●), 1.0% (■), 2.0% (◆), (b) saline soil: 0% (▽), 0.5% (○), 1.0% (□), 2.0% (◇); bars indicate standard deviation.

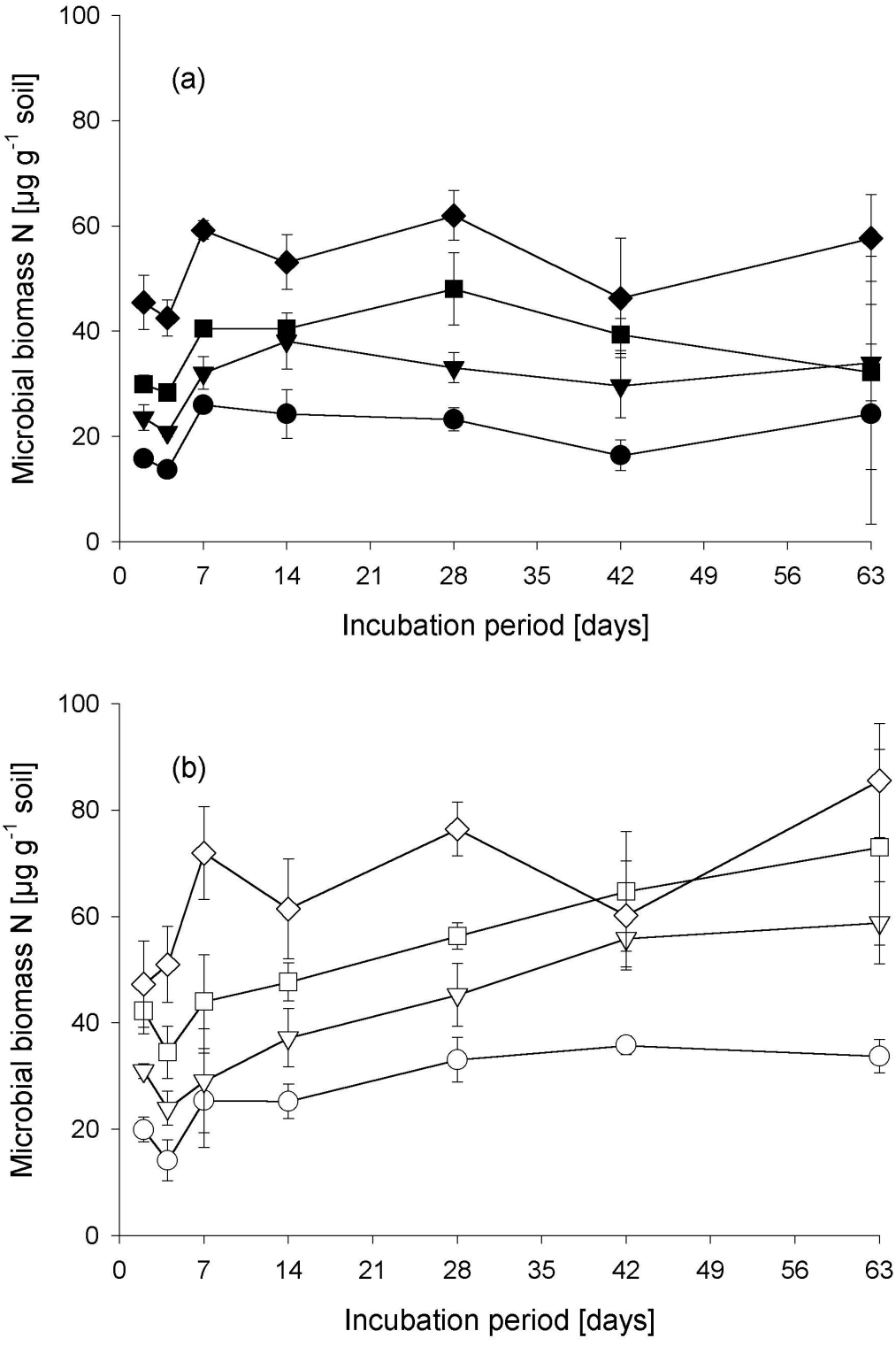

Fig. 3

Microbial biomass N content in the treatments without and with different addition rates of sugarcane filter cake (0.5, 1.0, and 2.%) at 7 sampling dates during a 63-day incubation at 30°C; (a) non-saline soil: 0% (▼), 0.5% (●), 1.0% (■), 2.0% (◆), (b) saline soil: 0% (▽), 0.5% (○), 1.0% (□), 2.0% (◇); bars indicate standard deviation.

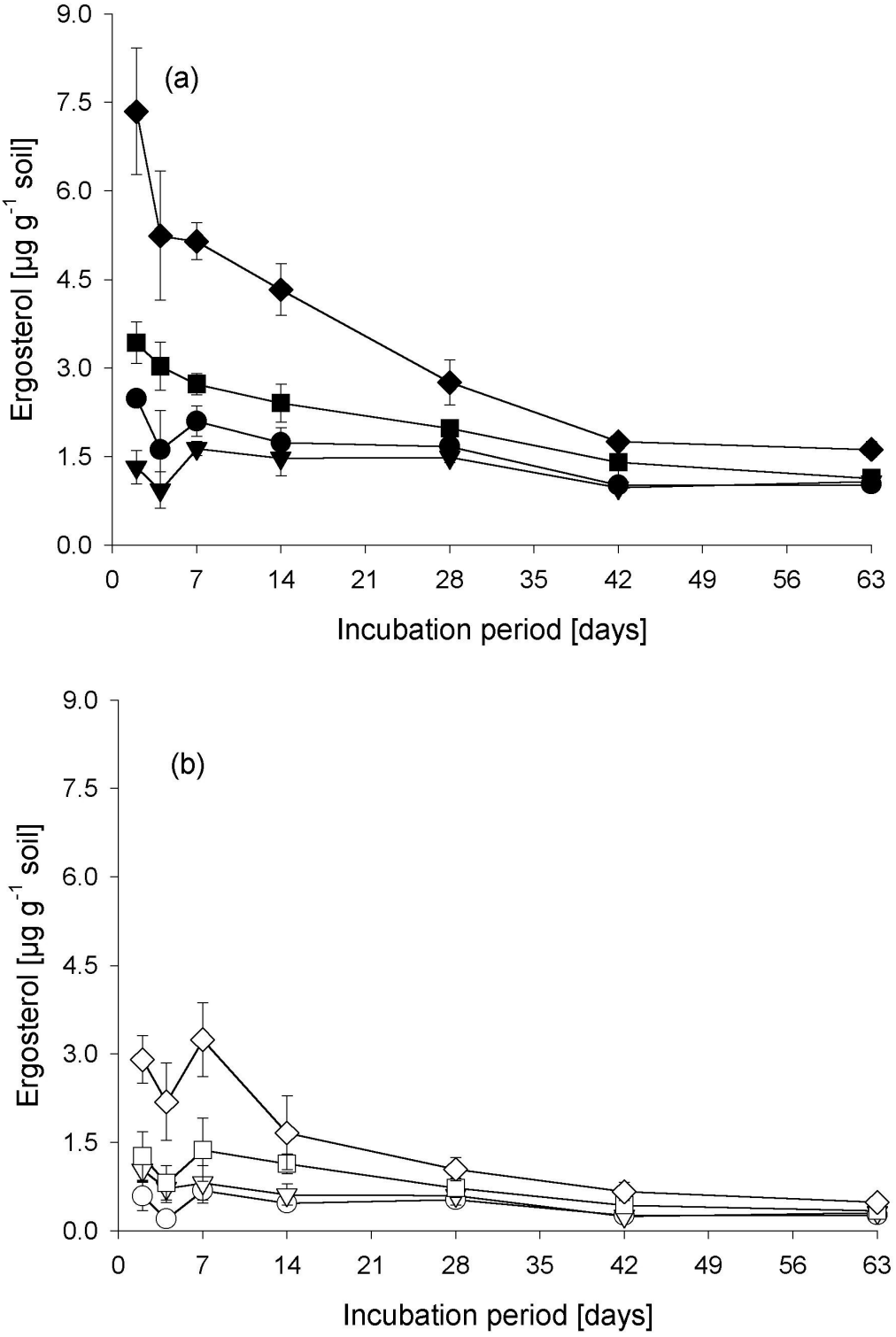

Fig. 4

Ergosterol content in the treatments without and with different addition rates of sugarcane filter cake (0.5, 1.0, and 2.%) at 7 sampling dates during a 63-day incubation at 30°C; (a) non-saline soil: 0% (▼), 0.5% (●), 1.0% (■), 2.0% (◆), (b) saline soil: 0% (▽), 0.5% (○), 1.0% (□), 2.0% (◇); bars indicate standard deviation.

2.5 Discussion

Virtually no nitrate was formed in the saline acidic soil. This inhibition of nitrification is consistent with the results of Pathak and Rao (1998) under strongly saline alkaline conditions. The addition of sugarcane filter cake led to immobilisation of inorganic N in both soils. The amount of immobilised N was similar to the increase in microbial biomass N in the non-saline soil, but it was considerably higher in the saline soil. Increasing N immobilisation was also observed by Luna-Guido et al. (2003) with increasing salinity in alkaline Mexican soils after the addition of maize residues. The stronger immobilisation of N in the saline soil is in line with the considerably wider ratio of CO_2-C to net N mineralised released during the incubation in the non-amended control soil. This ratio was 29 in the saline control soil, but only 16 in the non-saline control soil.

The immobilisation of N after adding sugarcane filter cake was not expected, because the total C-to-total N ratio of this material was below 13 and the organic C-to-organic N ratio in the 0.5 K_2SO_4 extract was even lower at 9.2. The sugarcane filter cake consists apparently of two main fractions: (1) One fraction consists of easily decomposable, but N-poor C components. (2) Another fraction consists of highly soluble, N-rich, but rather recalcitrant organic material. Consequently, neither low C-to-N ratios nor high concentrations of soluble material necessarily indicate good N availability to soil microorganisms, especially under saline conditions. This is in contrast to many other experiments, e.g. with mulch material (Seneviratne et al., 1998) and farmyard manure (Wichern et al., 1994).

In contrast to nitrification and N immobilisation, the net increases in microbial biomass N, microbial biomass C and CO_2 production were similar in both soils after the addition of sugar cane filter cake. The increase in CO_2 production was linearly related to the amount of filter cake added and not affected by the differences in the initial microbial biomass C content. Also the increases in microbial biomass C and biomass N were linearly related to the amount of filter cake added, but this increase was slightly higher for both properties in the saline soil. The hypothesis that the incorporation of a substrate depends on the ratio of substrate to the initial microbial biomass (Witter and Kanal, 1998) is apparently also true for complex organic substrates and small differences in microbial biomass.

The lower content of microbial biomass C in the non-saline grassland soil in comparison to the vegetation-free saline soil seems to be related to the lower content in

soil organic matter. These two properties were considerably lower than those observed by Sardinha et al. (2003) at a neighbouring non-saline site. This apparent spatial heterogeneity of the floodplain can only be explained by differences in the land-use history, because all other chemical and physical properties are within the range described by Sardinha et al. (2003). However, the saline control soil exhibited a lower microbial biomass C-to-soil organic C ratio (0.44 versus 0.70%) and a higher metabolic quotient qCO_2 (124 versus 94 mg CO_2-C g^{-1} microbial biomass C d^{-1}) in comparison to the non-saline control soil on the basis of the mean data shown in Table 1. The level of the microbial biomass C-to-soil organic C ratio, which is an index for the C availability to soil microorganisms, was generally low (Anderson and Joergensen, 1997). The level of the qCO_2, which is an index for the age structure of the soil microbial community, was generally high (Anderson and Joergensen, 1997). Consequently, both indices indicate that the soil microbial community suffers generally from stress by acidity, which is intensified by salinity.

In contrast to the present results, no increase in microbial biomass was observed by Dee et al. (2003) in a 56-day incubation experiment carried out with 1 and 2% addition rates of sugarcane filter cake using two acidic South African Luvisols. Dee et al. (2003) explained their observation as a result of changes in the microbial community structure. In the present experiment, the strongest increase in microbial biomass C occurred within the first 2 days after the amendment. This might indicate that the filter cake was heavily colonised by microorganisms before adding to the soil as suggested by Potthoff et al. (2001) for wheat straw and Flessa et al. (2002) for rye grass. However, this hypothesis contrasts the observation that the addition of different filter cake amounts did not affect the average soil-specific ergosterol-to-microbial biomass C ratio. This ratio can be used as an index for the fungal part of the total microbial biomass (Djajakirana et al., 1996). If those organisms initially colonising the substrate had a dominant role, the ergosterol-to-microbial biomass C ratio should have been developed similarly in the amendment treatments. However, the absolute increase in ergosterol in the saline soil was on average only half that in the non-saline soil. In contrast to microbial biomass C, the increase in ergosterol content not only reacted differently to salinity, but also showed strong temporal changes during the incubation. A strong initial increase after adding the filter cake was followed by a rapid decline, contrasting the view that large amounts of ergosterol are accumulated in dead fungal tissue (Zhao et al., 2005). However, it remains uncertain whether these current changes in the ergosterol content and in the

ergosterol-to-microbial biomass C ratio are due to changes in the ratio of fungal-to-bacterial biomass, due to changes in the fungal community structure, or due to changes in the composition of fungal membranes.

Ergosterol is an important component of fungal cell membranes responsible for their stability (Weete and Weber, 1980). The ergosterol concentration in cultivated microfungi is highly variable depending on species and nutritional status (Newell et al., 1987; Djajakirana et al., 1996). However, our knowledge of the 'in situ' variability of ergosterol concentrations in soil fungi, even under laboratory incubation conditions, is still restricted (Montgomery et al., 2000). The ergosterol-to-microbial biomass C ratio ranges from 0.1% in wetland soils (Djajakirana et al., 1996) to more than 3% in litter layers (Smolander et al. 1994), i.e. in situations with low or strong fungal dominance. If the ergosterol content is recalculated into fungal biomass C by multiplication by 90 (Djajakirana et al., 1996), fungi represent on average only 28% of total microbial biomass C in the saline soil and 87% in the non-saline soil. These values are reasonably in the range obtained by other methods, e.g. the selective inhibition technique (Blagodatskaya and Anderson, 1998).

2.6 Conclusions

In the saline soil without amendment, nitrification was inhibited, the microbial biomass C-to-soil organic C ratio was reduced, and the metabolic quotient qCO_2 was increased. These results revealed that salinity intensified stress to the soil microbial community under acidic conditions. The lower ergosterol-to-microbial biomass C ratio in the saline soil indicates that especially the fungal community was affected by salinity. The addition of sugarcane filter cake led to immobilisation of inorganic N in both soils. This immobilisation was not expected, because the total C-to-total N ratio of the filter cake was below 13 and the organic C-to-organic N ratio in the 0.5 K_2SO_4 extract of this material was even lower at 9.2. The immobilisation was considerably higher in the saline soil than in the non-saline soil. The N-immobilisation capacity of sugarcane filter cake should be considered when this material is applied to arable sites at high rations. It should be tested whether a mixture of sugarcane filter cake with other organic materials, such as biogenic waste compost or animal manure could improve the behaviour of sugarcane filter cake in soil.

2.7 Acknowledgements

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2.8 References

Anderson, T.-H., Joergensen, R.G., 1997. Relationship between SIR and FE estimates of microbial biomass C in deciduous forest soils at different pH. Soil Biol. Biochem. 29, 1033-1042.

Badole, S.B., More, S.D., Raut, M.M., 2001. Residual effect of different organic and inorganic nutrient sources on yield and quality of groundnut. Ann. Plant Physiol. 15, 54-57.

Badran, R.A.M., 1994. Cellulolytic activity of some cellulose-decomposing fungi in salinized soils. Acta Mycol. 29, 245-221.

Bailly, F., Mueller, K., Nieder, R., Schön, H.-G., 1998. World Reference Base for Soil Resources. Bezugsgrundlagen der Boden-Resourcen der Erde (WRB). Techn. Univ. Braunschweig / Fachhochschule Osnabrück.

Batra, L., Manna, M.C., 1997. Dehydrogenase activity and microbial biomass carbon in salt-affected soils of semiarid and arid regions. Arid Soil Res. Rehabil. 11, 295-303.

Blagodatskaya, E.V., Anderson, T.-H., 1998. Interactive effects of pH and substrate quality on the fungal-to-bacterial ration and qCO_2 of microbial communities in forest soils. Soil Biol. Biochem. 30, 1269-1274.

Brookes, P.C., Landman, A., Pruden, G., Jenkinson, D.S., 1985. Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method for measuring microbial biomass nitrogen in soil. Soil Biol. Biochem. 17, 837-842.

Chen Y., Avnimelech, Y., 1986. The role of organic matter in modern agriculture. Martinus Nijhoff Publishers, Dordrecht.

Chander, K., Dyckmans, J., Joergensen, R.G., Meyer, B., Raubuch, M., 2001. Different sources of heavy metals and their long-term effects on soil microbial properties. Biol. Fertil. Soils 34, 241-247.

Dee, B.M., Haynes, R.J., Graham, M.H., 2003. Changes in soil acidity and the size and activity of the microbial biomass in response to the addition of sugar mill wastes. Biol. Fertil. Soils 37, 47-54.

Djajakirana, G., Joergensen, R.G., Meyer, B., 1996. Ergosterol and microbial biomass relationship in soil. Biol. Fertil. Soils 22, 299-304.

Flessa, H., Potthoff, M., Loftfield, N., 2002. Laboratory estimates of CO_2 and N_2O emissions following surface application of grass mulch: importance of indigenous microflora of mulch. Soil Biol. Biochem. 34, 875-879.

He, Z.L., Yang, X.E., Baligar, V.C., Calvert, D.V., 2003. Microbiological and biochemical indexing systems for assessing quality of acid soils. Adv. Agron. 78, 89-138.

Heinrichs, H., Brumsack, H.J., Loftfield, N., König, N., 1986. Verbessertes Druckaufschlusssystem für biologische und anorganische Materialien. Z. Pflanzenernähr. Bodenk. 149, 350-353.

Harper, S.H.T., Lynch, J.M., 1985. Colonization and decomposition of straw by fungi. Trans. Brit. Myc. Soc. 85, 655-661.

Joergensen, R.G., 1995. Die quantitative Bestimmung der mikrobiellen Biomasse in Böden mit der Chloroform-Fumigations-Extraktions-Methode. Gött. Bodenkundl. Ber. 104, 1-229.

Luna-Guido, M.L., Vega-Estrada, J., Ponce-Mendoza, A., Hernandez-Hernandez, H., Montes-Horcasitas, M.-C., Vaca-Mier, M., Dendooven, L., 2003. Mineralization of ^{14}C-labelled maize in alkaline saline soils. Plant Soil 250, 29-38.

Muralidharan, P., Rattan, R.K., 2001. Effect of lime and phosphorus application on quantity and intensity parameters of aluminium and its uptake by rice in acid saline soils. J. Indian Soc. Soil Sci. 49, 124-129.

Montgomery, H.J., Monreal, C.M., Young, J.C., Seifert, K.A., 2000. Determination of soil fungal biomass from soil ergosterol analyses. Soil Biol. Biochem. 32, 1207-1217.

Newell, S.Y., Miller, J.D., Fallon, R.D., 1987. Ergosterol content of salt-marsh fungi: effect of growth conditions and mycelial age. Mycologia 79, 688-695.

Pankhurst, C.E., Yu, S., Hawke, B.G., Harch, B.D., 2001. Capacity of fatty acid profiles and substrate utilization patters to describe differences in soil microbial communities associated with increased salinity or alkalinity at three locations in South Australia. Biol. Fertil. Soils 33, 204-217.

Pathak, H., Rao, D.L.N., 1998. Carbon and nitrogen mineralisation from added organic matter in saline and alkali soils. Soil Biol. Biochem. 30, 695-702.

Potthoff, M., Joergensen, R.G., Wolters, V., 2001. Short-term effects of earthworm activity and straw amendment on the microbial C and N turnover in a remoistened arable soil after summer drought. Soil Biol. Biochem. 33, 583-591.

Rietz, D.N., Haynes, R.J., 2003. Effects of irrigation-induced salinity and sodicity on soil microbial activity. Soil Biol. Biochem. 35, 845-854.

Sardinha, M., Müller, T., Schmeisky, H., Joergensen, R.G., 2003. Microbial performance in a temperate floodplain soil along a salinity gradient. Appl. Soil Ecol. 23, 237-244.

Schlichting, E., Blume, H.P., Stahr, K., 1995. Bodenkundliches Praktikum. 2nd Edition. Blackwell, Berlin.

Schmeisky, H., Podlacha, G., 2000. Natural revegetation of saline waste dumps – drought tolerant specialists and halophytes. Landscape Urban Plan. 51, 159-163.

Seneviratne, G., Van Holm, L.H.J., Kulasooriya, S.A., 1998. Quality of different mulch materials and their decomposition and N release under low moisture regimes. Biol. Fertil. Soils 26, 136-140.

Smolander, A., Kurka, A., Kitunen, V., Mälkönen, E., 1994. Microbial biomass C and N, and respiratory activity in soil of repeatedly limed and N- and P-fertilized Norway spruce stands. Soil Biol. Biochem. 26, 957-962.

Swift, M.J., Heal, O.W., Anderson, J.M., 1979 Decomposition in Terrestrial Ecosystems. Blackwell, Oxford.

Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. Soil Biol. Biochem. 19, 703-707.

Weete, J.D., Weber, D.J., 1980. Lipid biochemistry of fungi and other organisms. Plenum Publishing, New York.

Westhus, W., Fritzlar, F., Pusch, J., Van Elsen, T., Andres, C., 1997. Binnensalzstellen in Thüringen - Situationen, Gefährdung und Schutz. Naturschutz-Report 12, Thüringer Landesanstalt für Umwelt, Jena, 1-193.

Wichern, F., Müller, T., Joergensen, R.G., Buerkert, A., 2004. Effects of manure quality and application forms on soil C and N turnover of a subtropical oasis soil under laboratory conditions. Biol. Fertil. Soils 39, 165-171.

Witter, E., Kanal, A., 1998. Characteristics of the soil microbial biomass in soils from a long-term field experiment with different levels of C input. Applied Soil Ecology 10, 37-49.

Wu, J., Joergensen, R.G., Pommerening, B., Chaussod, R., Brookes, P.C., 1990. Measurement of soil microbial biomass C by fumigation-extraction - an automated procedure. Soil Biol. Biochem. 22, 1167-1169.

Xavier, S., Lonsane, B.K., 1994. Factors influencing fungal degradation of total soluble carbohydrates in sugarcane-pressmud under solid-state fermentation. Process Biochem. 29, 295-301.

Zahran, H.H., 1997. Diversity, adaption and activity of the bacterial flora in saline environments. Biol. Fertil. Soils 25, 211-223.

Zhao, X.R., Lin, Q., Brookes, P.C., 2005. Does soil ergosterol concentration provide a reliable estimate of soil fungal biomass? Soil Biol. Biochem. 37, 311-317.

Chapter 3 - Soil microbial response to sugarcane filter cake and biogenic waste compost

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3.1 Abstract

An incubation experiment was carried out to examine the N immobilizing effect of sugarcane filter cake (C/N ratio of 12.4) and to prove whether mixing it with compost (C/N ratio of 10.5) has any synergistic effects on C and N mineralization after incorporation into the soil. Approximately 19% of the compost C added and 37% of the filter cake C were evolved as CO_2, assuming that the amendments had no effects on the decomposition of soil organic C. However, only 28% of the added filter cake was lost according to the total C and $\delta^{13}C$ values. Filter cake and compost contained initially significant concentrations of inorganic N, which was nearly completely immobilized between day 7 and 14 of the incubation in most cases. After day 14, N re-mineralization occurred at an average rate of 0.73 µg N g^{-1} soil d^{-1} in most amendment treatments, paralleling the N mineralization rate of the non-amended control without significant difference. No significant net N mineralization from the amendment N occurred in any of the amendment treatments in comparison to the control. The addition of compost and filter cake resulted in a linear increase in microbial biomass C with increasing amounts of C added. This increase was not affected by differences in substrate quality, especially the three times larger content of K_2SO_4 extractable organic C in the sugarcane filter cake. In most amendment treatments, microbial biomass C and biomass N increased until the end of the incubation. No synergistic effects could be observed in the mixture treatments of compost and sugarcane filter cake.

Keywords: Microbial biomass C, Microbial biomass N, CO_2 evolution, N re-mineralization, $\delta^{13}C$

3.2 Introduction

Soil organic matter (SOM) is crucial for maintaining soil fertility, due to the positive impact on biological, chemical and physical properties of soils. Many soils in Europe (*Jones* et al., 2005a) and especially in semi-arid tropical regions are characterized by low carbon concentration, and this has negative consequences for crop yield (*Khan* and *Joergensen*, 2006). In the last decade, the function of SOM as a C sink has been given increased attention in numerous investigations, due to the threat of global warming (*Lal*, 2004; *Jones* et al., 2005b). An important component of cropland management for the maintenance or enhancement of SOM concentration is the addition of secondary organic fertilizers such as biogenic waste compost, especially for farms with little or no livestock (*Leifeld* et al., 2002; *Speir* et al., 2004; *Quintern* et al., 2006). Additionally, composting of biogenic municipal household waste is an important tool of public waste management systems for recycling nutrients and saving disposal space (*Fricke* et al., 2003; *Gall* et al., 2004).

In countries with sugarcane based sugar industry, such as Pakistan or India, sugarcane filter cake has the potential to serve as an important C source (*Badole* et al., 2001). However, strong immobilization of inorganic N has been observed after adding sugarcane filter cake to moderately acidic soils at pH 5.5 in $CaCl_2$ (*Rasul* et al., 2006). This result was rather unexpected, because the total C-to-total N ratio of the filter cake was below 13 and the organic C-to-organic N ratio in the 0.5 K_2SO_4 extract of this material was not much different. No N immobilization should occur in soil after adding substrates with a total C-to-total N ratio less than 15 (*Powlson* et al. 2001). This is a vast oversimplification, as plant N can be stabilized by association with ligninic or phenolic components during decomposition (*Powlson* et al. 2001), creating a pool of soluble organic N high in molecular weight and rich in humic substances (Jones et al., 2004).

The inorganic N content, i.e. the difference between initial immobilization into microbial residues and the following re-mineralization (*Mueller* et al., 1998), was considerably higher in the saline soil than in the non-saline soil at the end of the incubation experiment (*Rasul* et al., 2006). They concluded that the sugarcane filter cake consisted of two main fractions: (1) one fraction of easily decomposable, but N-poor C components, (2) another fraction of highly soluble, N-rich, but rather recalcitrant organic material. The first pool leads to rapid immobilization of inorganic N present in

soils, the second pool is inaccessible for the microorganisms that have colonized the filter cake on the dump. Biogenic composts contain highly diverse microbial communities (*Tebbe*, 2002; *Franke-Wittle* et al., 2005) and may contain microorganisms that are able to decompose the recalcitrant fraction of the sugarcane filter cake at relatively higher rates than those surviving the harsh conditions on the factory dumps. The processing conditions of organic wastes have a strong impact on C and N mineralization after incorporation into soil (*Cambardella* et al., 2003): For these reasons, our objective was to investigate whether blending of sugarcane filter cake with biogenic waste compost induces synergistic effects, increasing the decomposition rate of the sugarcane filter cake after addition to a non-saline soil.

Table 1

Concentrations of elements on a dry matter basis and elemental ratios in sugarcane filter cake and biogenic compost

	Filter cake	Compost
Total C (mg g^{-1})	420	221
$\delta^{13}C$ (‰)	13.3	26.3
Extractable organic C (mg g^{-1})	19	5.4
Total N (mg g^{-1})	34	21
Extractable total N (mg g^{-1})	2.2	1.2
Total P (mg g^{-1})	12.3	4.0
Total S (mg g^{-1})	3.9	3.2
K (mg g^{-1})	11.7	14
Mg (mg g^{-1})	3.9	10
Ca (mg g^{-1})	23.4	38
Total C/N	12.4	10.5
Extractable organic C/N	12.4	13.4
Total C/P	34	55
Total C/S	108	69

3.3 Materials and Methods

3.3.1 Soil and amendments

The soil used for the experiment was taken as field moist bulk sample at 0-20 cm depth from the site Allerberg, 10 km to the south of Göttingen, Germany. The soil had a pH in water of 6.2, a cation exchange capacity of 130 $\mu mol_C\ g^{-1}$ soil, with a base saturation of 65%. The soil contained 74% sand, 14% silt, and 12% clay, 8.2 mg organic C, 0.8 mg total N and 0.58 mg total P g^{-1} soil. The soil was classified as Eutric Cambisol according to the FAO-WRB classification system, derived from New Red Sandstone. 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 (*Rasul* et al., 2006). Biogenic municipal waste compost was taken from the compost plant in the vicinity of Witzenhausen (*Gattinger* et al., 2004). The elemental composition of sugarcane filter cake and compost is shown in Table 1).

3.3.2 Incubation procedure

The experiment was carried out with the following seven treatments in triplicate: (1) non-amended control soil, (2) +1.0% (dry weight / dry weight) sugarcane filter cake, (3) +2.0% filter cake, (4) +1.0% compost, (5) +2.0% compost, (6) +1.0%+1.0 mixture of filter cake and compost, and (7) 2.0%+2.0% mixture of filter cake and compost. The amounts of C added with the different treatments are shown in Table 2. The addition rates were in the range used in laboratory incubation experiments (*Dee* et al., 2003) or field experiments (*Badole* et al., 2001; *Quintern* et al., 2006). The addition rates were roughly equivalent to amounts of between 10 – 40 t dry weight ha^{-1} assuming a bulk density of 1.5 g cm^{-3} and a plough layer of 30 cm depth. For each treatment, 1 kg (on an oven-dry basis) of soil (< 2 mm) at 50% water holding capacity plus the different amendments were weighed into 3 l incubation vessels and incubated for 56 days at 30˚C in the dark. The amendments were thoroughly mixed with the soil immediately before the incubation was started. The CO_2 evolved was absorbed in 20 to 60 ml 2 M NaOH solution and back titrated to pH 8.3 with 1 M HCl after addition of 0.5 M $BaCl_2$ solution. The NaOH solution was changed after 2, 5, 8, and 14 days and thereafter

weekly. Soil samples of 100 g fresh-weight were taken at day 0, and after 5, 14, 28, and 56 days for the determination of microbial biomass and inorganic N concentration (NH_4-N and NO_3-N). This means that 600 g moist soil were left for the final incubation period from day 28 to day 56, considering that the initial sample was removed before the soil was transferred to the incubation vessels.

3.3.3 Analytical procedures

Microbial biomass C and biomass N were estimated by fumigation-extraction (*Brookes* et al., 1985; *Vance* et al., 1987). Two portions of 25 g moist soil were taken from the 100 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 100 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 = (organic C extracted from fumigated soils) - (organic C extracted from non-fumigated soils) and k_{EC} = 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 = (total N extracted from fumigated soils) - (total N extracted from non-fumigated soils) and k_{EN} = 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.

Total C in soil was measured after combustion using a Vario Max elemental analyzer (Elementar, Hanau, Germany). $\delta^{13}C$ values of solid samples were measured on a Delta plus IRMS 251 (Finnigan Mat, Bremen, Germany) after combustion using an NA 1500 elemental analyzer (Carlo Erba, Milan, Italy).

3.3.4 Calculations and statistical analysis

The part of C_4-sugarcane filter cake derived C in total C was calculated from the $\delta^{13}C$ data using the following equation:

$$C_4\text{-}Csample = C_T sample \times \frac{\delta^{13}Csample - \delta^{13}C - C_3 reference}{\delta^{13}C - C_4 substrate - \delta^{13}C - C_3 reference} \quad (1)$$

$$C_3\text{-}Csample = C_T sample - C_4\text{-}Csample \quad (2)$$

The average of the δ^{13}C-values in the control soil and the pure compost treatments was used as $\delta^{13}C_3 reference$. Soil and compost did not differ significantly in their δ^{13}C-values. The results presented in the tables are arithmetic means and expressed on an oven-dry basis (about 24 h at 105°C). The significance of experimental effects on K_2SO_4 extractable organic C and organic N as well as that on microbial biomass C and on biomass N was tested by a one-way ANOVA with amendments as independent factor and sampling day as repeated measures. The significance of experimental effects on the sum of CO_2 production and net N mineralization was analyzed by means of a one-way ANOVA using the Scheffé post-hoc test, which is robust against the violation of normality and inhomogeneity of the variances (StatView Reference Manual, SAS Inst. Inc.). All statistical analyses were performed using StatView 5.0 (SAS Inst. Inc.).

3.4 Results

The addition of sugarcane filter cake and compost increased the CO_2 evolution rate during the 56-d incubation period markedly (Table 2). Approximately 37% of the filter cake C added and 19% of the compost C were evolved as CO_2, assuming that the amendments had no effects on the decomposition of soil organic C. In the 1%+1% mixture treatment, the CO_2 production corresponded to the weighted average of the two pure components, but it was lower in the 2%+2% mixture treatment. On average, 72% of the added filter cake was recovered according to the total C and δ^{13}C values (Table 3), i.e. 28% was lost.

Filter cake and compost contained initially significant concentrations of inorganic N, which led to higher inorganic N levels in comparison with the control. Except for the pure compost treatments, this inorganic N was nearly completely immobilized between day 7 and 14 of the incubation (Fig. 1a). After day 14, N re-mineralization occurred at an average rate of 0.73 µg N g^{-1} soil d^{-1} in most amendment treatments, paralleling the N mineralization rate of the non-amended control without significant difference. Exceptions were the 1%+1% mixture treatment with a significantly larger N re-

mineralization rate of 1.59 µg N g^{-1} soil d^{-1} and the 2% filter cake treatment with a significantly lower N re-mineralization rate of 0.29 µg N g^{-1} soil d^{-1}. No significant net N mineralization from the amendment N occurred in any of the amendment treatments in comparison to the control. In the 2% filter cake and 2%+2% mixture treatments, the contents of inorganic N remained at the end of the incubation even below the initial values.

Table 2

Amounts of C added with the different treatments, CO_2-C evolved and inorganic N (NH_4-N + NO_3-N) mineralised during a 56-d incubation period at 30ºC

Treatment	C added (mg g^{-1})	CO_2-C (µg g^{-1})	Inorganic N (µg g^{-1})
Control	0.00	600 e	42 a
1% filter cake	4.20	2240 b	10 b
2% filter cake	8.40	3600 a	-19 c
1% compost	2.21	1050 d	40 a
2% compost	4.42	1420 c	49 a
1%+1% mixture	6.41	2480 b	33 a
2%+2% mixture	12.82	3710 a	-27 c
CV (± %)		3.9	21

Different letters indicate a significant difference ($P < 0.05$, Scheffé, n = 3); CV = mean coefficient of variation between replicate measurements.

The addition of organic amendments increased the concentration of 0.5 M K_2SO_4 extractable organic C and organic N with increasing addition rate (Table 4). In the mixture treatments, the content of 0.5 M K_2SO_4 extractable organic C and organic N corresponded to the weighted average of the two pure components. The C/N ratio of extractable SOM varied around a mean value of 11 in the filter cake treatments and ranged between 19 and 27 in the control and pure compost treatments, respectively. During the incubation, the concentration of 0.5 M K_2SO_4 extractable organic C (Fig. 1b)

and organic N decreased rapidly during the first five days and remained roughly constant during the rest of the incubation.

Table 3

Total C, $\delta^{13}C$ and sugarcane filter cake recovered at the end of the 56-d incubation at 30°C

	Total C (mg g^{-1} soil)	$\delta^{13}C$ (‰)	Filter cake recovered (% added)
Control	8.8 c	-26.2 c	
1% filter cake	10.8 bc	-22.4 ab	78
2% filter cake	11.9 b	-20.5 a	64
1%+1% mixture	12.7 b	-22.9 b	78
2%+2% mixture	16.3 a	-21.5 ab	72
CV (± %)	8.3	2.8	13

Different letters indicate a significant difference ($P < 0.05$, Scheffé, n = 3); CV = mean coefficient of variation between replicate measurements.

The addition of both compost and filter cake proportionately increased the contents of microbial biomass C with increasing addition rate (Table 3). This increase was proportionate to the amount of C added, resulting in a linear increase in microbial biomass C with increasing amounts of C added (r = 0.98, n = 6, $P < 0.01$, Fig. 2a). In the control treatment, microbial biomass C remained constant throughout the incubation (Fig. 2b). In most amendment treatments, microbial biomass C increased with considerable variation between the sampling days and between the replicate samples until the end of the incubation. Microbial biomass N followed biomass C, with a C/N ratio of 4.5 in the pure filter cake treatments and roughly 5.5 in the other treatments (Table 3).

Table 4

Main effects of the experiment on extractable organic matter and microbial properties during a 56-d incubation period at 30ºC, mean values of the 7 treatments over the 5 sampling days and mean values of 5 sampling days over the 7 treatments

	0.5 M K_2SO_4 extractable organic			Microbial biomass		
	C ($\mu g\ g^{-1}$)	N ($\mu g\ g^{-1}$)	C/N	C ($\mu g\ g^{-1}$)	N ($\mu g\ g^{-1}$)	C/N
Control	59 ± 1	2.5 ±0.3	27 ±2	204 ±10	38 ±2	5.5 ±0.3
1% filter cake	113 ±15	9.4 ±0.6	12 ±1	286 ± 9	66 ±4	4.5 ±0.2
2% filter cake	172 ±34	14.7 ±1.9	11 ±1	345 ±18	79 ±5	4.4 ±0.2
1% compost	85 ± 4	4.6 ±0.2	19 ±1	235 ±10	50 ±4	5.1 ±0.4
2% compost	119 ± 9	7.8 ±1.9	22 ±2	276 ±22	48 ±3	5.8 ±0.3
1%+1% mixture	138 ±18	13.8 ±1.3	11 ±1	299 ±12	58 ±3	5.4 ±0.4
2%+2% mixture	240 ±45	23.4 ±2.5	9 ±1	377 ±25	74 ±5	5.2 ±0.3
Day 0	260	17	21	250	50	5.2
Day 5	110	11	13	260	53	4.9
Day 14	110	8.1	17	270	57	5.2
Day 28	90	8.4	14	330	65	5.1
Day 56	90	9.9	14	330	71	5.2
Probability levels						
Treatment	<0.01	<0.01	<0.01	<0.01	<0.01	0.01
Sampling **d**ay	<0.01	<0.01	<0.01	<0.01	<0.01	0.82
T x **Sd**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
CV (± %)	9	18	16	13	13	13

± Standard error of mean (n = 3); CV = mean coefficient of variation between replicate measurements.

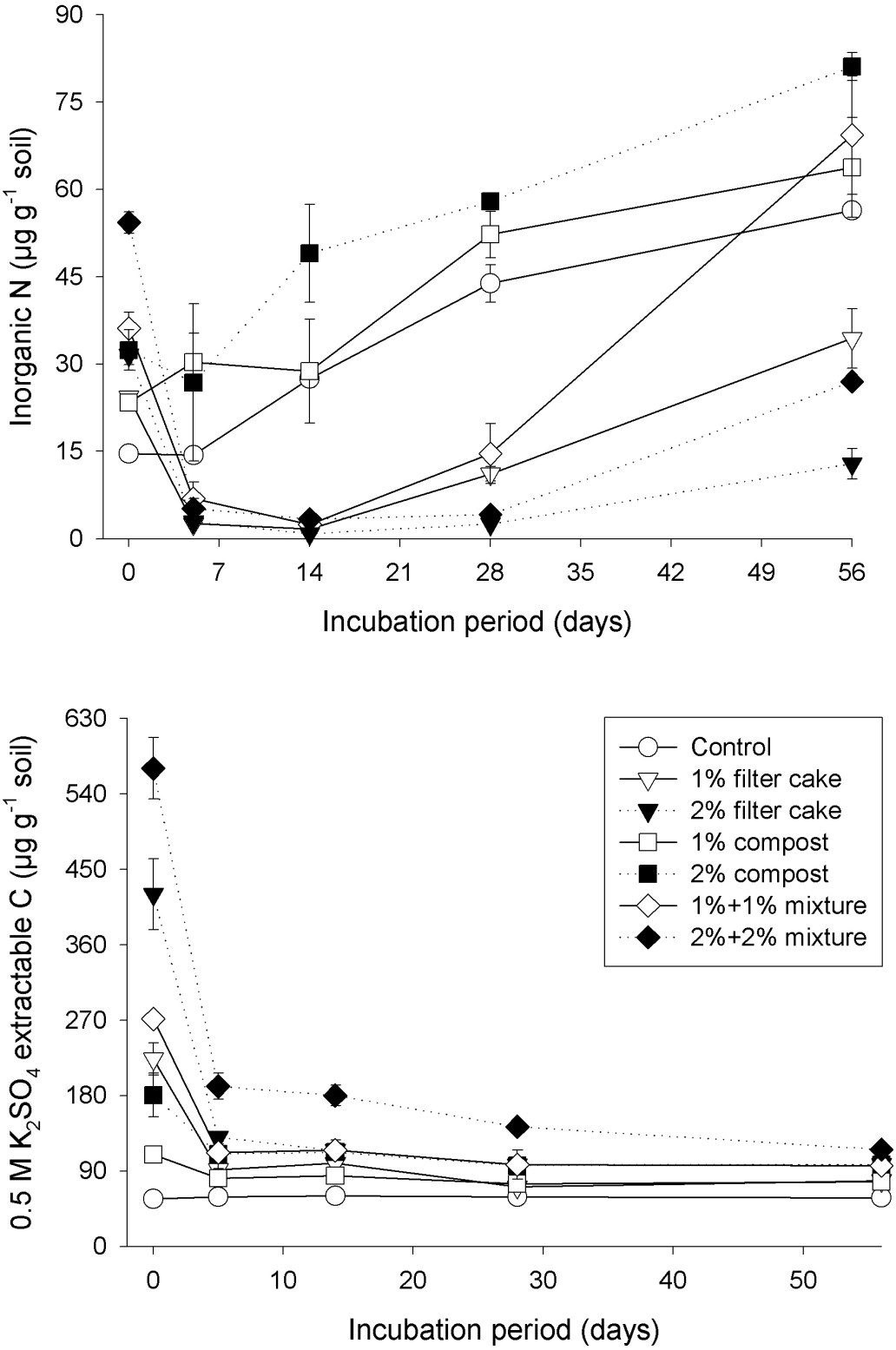
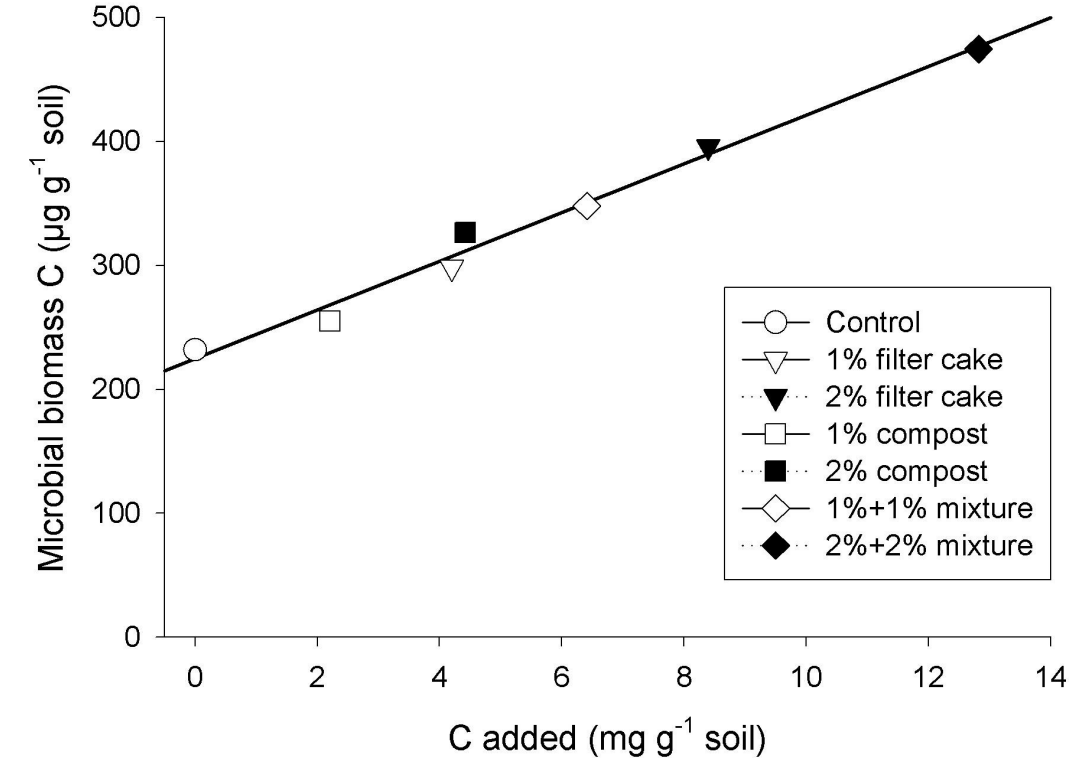


Fig. 1
Changes (a) in 0.5 M K_2SO_4 extractable inorganic N (NO_3-N + NH_4-N) and (b) in 0.5 M K_2SO_4 extractable organic C in the five treatments during a 56-d incubation at 30°C; bars indicate ± one standard deviation.



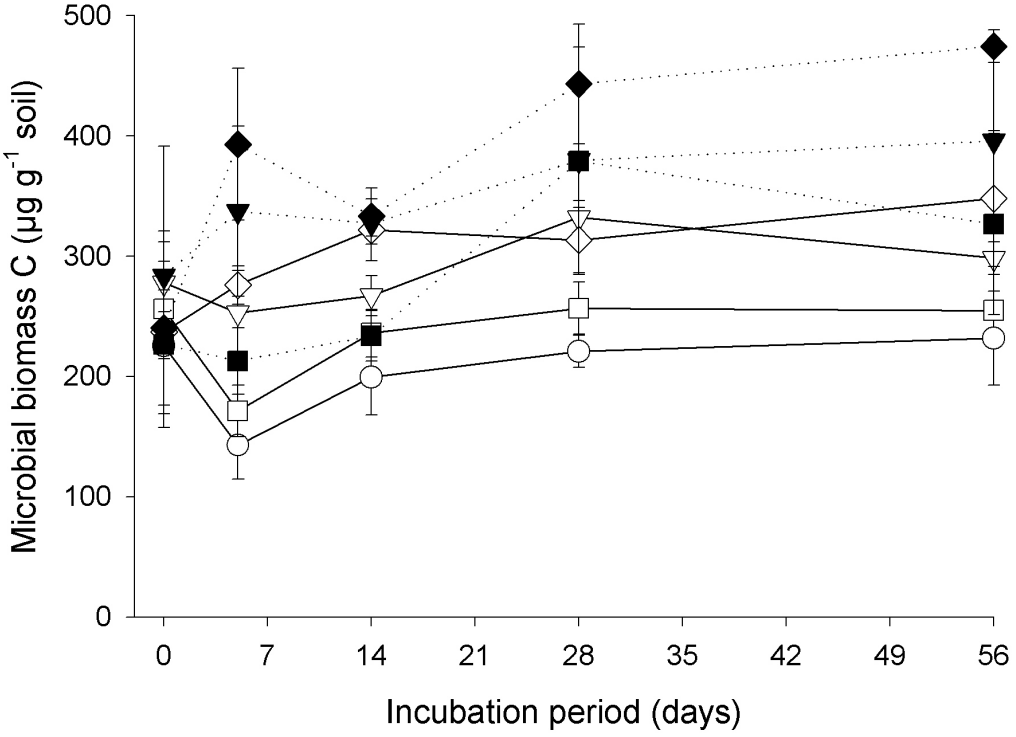


Fig. 2

(a) Linear relationship between microbial biomass C at day 56 and the amount of C added initially and (b) changes in microbial biomass C in the five treatments during a 56-d incubation at 30°C; bars indicate ± one standard deviation.

3.5 Discussion

Sugarcane filter cake leads to strong N immobilization in a neutral non-saline soil, although it contains high contents of total N and extractable organic N. These results are in agreement with those of *Rasul* et al. (2006). They are also in line with those of *Wichern* et al. (2004) investigating the mineralization of farmyard manure, which has a similar low C/N ratio to the present filter cake. Neither the C/N ratio of total SOM nor that of the extractable fraction are good indicators for N mineralization in complex organic amendments. A specific feature of the sugarcane filter cake is its high concentration of extractable organic C, which decreased as biological decomposition proceeded. The importance of extractable organic C for the immobilization of N has been shown repeatedly for immature or fresh farmyard manure (*Martin-Olmedo* and *Rees,* 1999; *Wichern* et al., 2004). However, also the decomposition of compost did not lead to a significant net N mineralization, despite a C/N ratio not much higher than that of the sugarcane filter cake. In contrast to the present net N mineralization rate, 1% compost addition led to a strong increase in plant growth during a 90-day pot experiment (*Muhammad* et al., 2007). A certain proportion of nutrients released during the decomposition of compost are apparently available to plants. However, complex interactions between plant growth and N release from compost cannot be monitored by incubation experiments.

A priming effect, i.e. an increased decomposition of native soil organic C might be an explanation for differences between the CO_2 evolution and the C recovered according to the total C and $\delta^{13}C$ values (*Kuzyakov* et al., 2000; *Zyakun* and *Dilly,* 2005). The addition of maize-cellulose resulted in a 100% increase in the evolution of native SOM-derived CO_2 evolution during a four-week incubation period (*Engelking* et al., 2007). However, methodological limitations are another explanation for the differences between the CO_2 evolved and the C recovered. The determination of $\delta^{13}C$ is usually very accurate, with coefficients of variation below 1% (*Engelking* et al., 2007), although the determination is carried out in small sub-samples. Also the determination of total C in SOM is regularly conducted as one of the most accurate methods in soil analysis, with coefficients of variation below 2% (*Becker*, 1992). However, in the present experiment, the distribution of total C, especially in the treatments with organic amendments, is too variable to allow an exact C balance at the end of the incubation. The measurement of CO_2 production by titration exhibited a slightly higher coefficient

of variation than the determination of $\delta^{13}C$ and total C, but this method records the total amount evolved during the incubation. Large differences in C concentration within the sugarcane filter cake are less likely, due to the thorough homogenization during milling.

The increase in microbial biomass was proportionate to the amounts of C added, although the two amendments differed in their composition, especially the concentration of extractable organic C. The combined addition of compost and filter cake has no synergistic effects. CO_2 evolution and N mineralization in the two mixture treatments could be predicted from the results of the pure amendments. There was no clear indication that the organic amendments added at higher rates, such as the 2%+2% mixture treatment, decompose differently than when added at lower rates. This finding is in contrast to the view of *Witter* and *Kanal* (1998) who reported a decrease in decomposition rate with increasing ratio of substrate C to microbial biomass C. The linear increase in microbial biomass C indicates that compost and sugarcane filter cake added new micro-sites for the microbial colonization of soil (*Wu* et al., 1993), independently of the autochthonous microbial community (*Flessa* et al., 2002).

Sugarcane filter cake is a potential source of SOM in many sub-tropical countries with soils low in organic matter. However, its N immobilization capacity is still of some concern. On the other hand, a temporal N immobilization caused by sugarcane during times of low crop demand might help to prevent leaching losses at irrigated cropland sites, thereby increasing the efficiency of nutrient use (*Wichern* et al., 2004).

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3.7 References

Badole, S.B., More, S.D., Raut, M.M. (2001): Residual effect of different organic and inorganic nutrient sources on yield and quality of groundnut. *Ann. Plant Physiol.* 15, 54-57.

Becker, K.-W. (1992): Ringanalyse auf Gesamt-C und Gesamt-N an Bodenproben. *Z. Pflanzenernähr. & Bodenk.*156, 191-192.

Brookes, P.C., Landman, A., Pruden, G., Jenkinson, D.S. (1985): Chloroform fumigation and the release of soil nitrogen: A rapid direct extraction method for measuring microbial biomass nitrogen in soil. *Soil Biol. Biochem.* 17, 837-842.

Cambardella, C.A., Richard, T.L., Russell, A. (2003): Compost mineralization in soil as a function of composting process conditions. *Eur. J. Soil Biol.* 39, 117-127.

Dee, B.M., Haynes, R.J., Graham, M.H. (2003): Changes in soil acidity and the size and activity of the microbial biomass in response to the addition of sugar mill wastes. *Biol. Fertil. Soils* 37, 47-54.

Engelking, B., Flessa, H., Joergensen, R.G. (2007): Formation and use of microbial biomass and microbial residues from maize cellulose and sugar cane saccharose. *Soil Biol. Biochem*. 39, 1888-1896.

Flessa, H., Potthoff, M., Loftfield, N. (2002): Greenhouse estimates of CO_2 and N_2O emissions following surface application of grass mulch: importance of indigenous microflora of mulch. *Soil Biol. Biochem.* 34, 875-879.

Franke-Whittle, I.H., Klammer, S.H., Insam, H. (2005): Design and application of an oligonucleotide microarray for the investigation of compost microbial communities. *J. Microbiol. Meth.* 62, 37-56.

Fricke, K., Goedecke, *H., Einzmann, U.* (2003): Die Getrenntsammlung und Verwertung von Bioabfällen – Bestandsaufnahme 2003. Die Zukunft der Getrenntsammlung von Bioabfällen. Schriftenreihe des ANS 44, Orbitverlag, Weimar, pp. 11-64.

Gall, R.J., Fiegl, L., Burroughs, J., *Miller, I.* (2004): Saving millions in landfilling costs. *BioCycle* 45, 22-23.

Gattinger, A., Bausenwein, U., Bruns, C. (2004): Microbial biomass and activity in composts of different composition and age. *J. Plant Nutr. Soil Sci.* 167, 556-561.

Joergensen, R.G., Mueller, T. (1996): The fumigation-extraction method to estimate soil microbial biomass: Calibration of the k_{EN} value. *Soil Biol. Biochem.* 28, 33-37.

Jones, C., McConnell, C., Coleman, K., Cox, P., Falloon, P., Jenkinson, D., Powlson, D. (2005b): Global climate change and soil carbon stocks; predictions from two contrasting models for the turnover of organic carbon in soil. *Global Change Biol.* 11, 154-166.

Jones, D.L., Shannon, D., Murphy, D.V., Farrar, J. (2004): Role of dissolved organic nitrogen (DON) in soil N cycling in grassland soils. *Soil Biol. Biochem.* 36, 749-756.

Jones, R.J.A., Hiederer, R., Rusco, E., Montanarella, L. (2005a): Estimating organic carbon in the soils of Europe for policy support. *Eur. J. Soil Sci.* 56, 655-671.

Khan, K.S., Joergensen, R.G. (2006): Microbial C, N, and P relationships in moisture stressed soils of Potohar, Pakistan. *J. Plant Nutr. Soil Sci.* 169, 494-500.

Kuzyakov, Y., Friedel, J.K., Stahr, K. (2000): Review of mechanisms and quantification of priming effects. *Soil Biol. Biochem.* 32, 1485-1498.

Lal, R. (2004): Agricultural activities and the global carbon cycle. *Nutr. Cyc. Agroecosyst.* 70, 103-116.

Leifeld, J., Siebert, S., Kögel-Knabner, I. (2002): Biological activity and organic matter mineralization of soils amended with biowaste composts. *J. Plant Nutr. Soil Sci.* 165, 151-159.

Martin-Olmedo, P., Rees, R.M. (1999): Short-term N availability in response to dissolved-organic-carbon from poultry manure, alone or in combination with cellulose. *Biol. Fertil. Soils* 29, 386-393.

Mueller, T., Jensen, L.S., Nielsen, N.E., Magid, J. (1998): Turnover of carbon and nitrogen in a sandy loam soil following incorporation of chopped maize plants, barley straw and blue grass in the field. *Soil Biol. Biochem.* 30, 561-571.

Muhammad, S., Müller, T., Joergensen, R.G. (2007): Soil amendments for stimulating microorganisms and maize growth in a saline Pakistani soil. *J. Plant Nutr. Soil Sci* (in press).

Powlson, D.S., Hirsch, P.R., Brookes, P.C. (2001): The role of soil microorganisms in soil organic matter conservation in the tropics. *Nutr. Cycl. Agroecosys.* 61, 41-51.

Quintern, M., Lein, M., Joergensen, R.G. (2006): Changes in soil biological quality indices after long-term addition of shredded shrubs and biogenic waste compost. *J. Plant Nutr. Soil Sci.* 169, 488-493.

Rasul, G., Appuhn, A., Müller, T., Joergensen, R.G. (2006): Salinity induced changes in the microbial use of sugar cane filter cake material under acidic soil conditions. *Appl. Soil Ecol.* 31, 1-10.

Speir, T.W., Horswell J., van Schaik, A.P., McLaren, R.G., Fietje, G. (2004): Composted biosolids enhance fertility of a sandy loam soil under dairy pasture. *Biol. Fertil. Soils* 40, 349-358.

Tebbe, C.C. (2002): DNA-based research uncovers composting microorganisms. *BioCycle* 43, 24-27.

Vance, E.D., Brookes, P.C., Jenkinson, D.S. (1987): An extraction method for measuring soil microbial biomass C. *Soil Biol. Biochem*. 19, 703-707.

Wichern, F., Müller, T., Joergensen, R.G., Buerkert, A. (2004): Effects of manure quality and application forms on soil C and N turnover of a subtropical oasis soil under laboratory conditions. *Biol. Fertil. Soils* 39, 165-171.

Witter, E., Kanal, A. (1998): Characteristics of the soil microbial biomass in soils from a long-term field experiment with different levels of C input. *Appl. Soil Ecol*. 10, 37-49.

Wu, J., Joergensen, R.G., Pommerening, B., Chaussod, R., Brookes, P.C. (1990): Measurement of soil microbial biomass-C by fumigation-extraction - an automated procedure. *Soil Biol. Biochem*. 22, 1167-1169.

Wu, J., Brookes, P.C., Jenkinson, D.S. (1993): Formation and destruction of microbial biomass during the decomposition of glucose and ryegrass in soil. *Soil Biol. Biochem.* 25, 1435-1441.

Zyakun, A., Dilly, O. (2005): Respiratory quotient and priming effect in an arable soil induced by glucose. *Appl. Biochem. Microbiol.* 41, 512-520.

Chapter 4 – Immobilization and mineralization of nitrogen during microbial use of sugarcane filter cake amended with glucose in a saline and alkaline soil

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4.1 Abstract

A 42-day 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. The relative percentage CO_2 evolved increased from 35% of the added C in the pure 0.5% filter cake treatment to 41% in the 0.5% filter cake +0.25% glucose treatment to 48% in the 0.5% filter cake +0.5% glucose treatment. The three different amendment treatments led to immediate increases in microbial biomass C and biomass N within 6 h, which persisted until the end of the incubation only in the pure filter cake treatment. 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 amendment treatments and was thus 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. Other unknown soil properties seem to form a soil-specific gate for the release of inorganic N.

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

4.2 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 soil lacking soil 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 of any inorganic N directly after the amendment of sugar cane 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 later disproportionately increased 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.

4.3 Materials and Methods

4.3.1 Soil and amendments

A bulk sample of 20 kg arable soil was taken at 0-15 cm depth from the Sargodha district, Punjab province (33.36°N and 73.07°E) of Pakistan during July 2002 (Muhammad et al. 2006). The soil was classified as Salic Solonetz according to the FAO-WRB (world

reference basis) classification system. The soil (standard deviation (SD) in brackets) had a pH in water of 8.23 (0.04), a $CaCO_3$ content of 16.1% (0.2) and 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 soil (SD in brackets) contained 25.2% (0.7) sand, 64.7% (1.1) silt, and 10.1% (0.5) clay, 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). The soil (SD in brackets) contained 87.8 (0.8) µg NO_3-N g^{-1} soil and 0.2 (0.1) µg NH_4-N g^{-1} soil extractable with 0.5 M K_2SO_4, 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 (Rasul et al. 2006). The sugarcane filter cake contained the following element and nutrient concentrations g^{-1} dry weight (SD in brackets): 440 (8.0) mg C, 34 mg (1.4) 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.

4.3.2 Incubation procedure

The soil was adjusted to 50% 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) +0.5% sugarcane filter cake, (3) + 0.5% sugarcane filter cake +0.25% glucose +8 mg $(NH_4)_2SO_4$ g^{-1} soil, and (4) 0.5% sugarcane filter cake +0.5% glucose +16 mg $(NH_4)_2SO_4$ g^{-1} soil. The organic amendments of treatments (2), (3), and (4) were equivalent to 2000, 3000, and 4000 µg C g^{-1} soil. For each treatment 200 g (oven-dry basis) quadruplicate samples of soil without or with the different amendments were placed into 3 l incubation vessels and incubated for 42 days at 30°C in the dark. The CO_2 evolved was absorbed in 1 M NaOH solution and analysed by back-titration with HCl after 2, 4, 8, 14, 21, 28, 35 and 42 days of incubation. Soil samples of 50 g oven dry weight were collected at 0, 14, 28 and 42 days after incubation and analyzed for different microbial biomass and activity indices.

4.3.3 Microbial biomass and K_2SO_4 extractable components

Microbial biomass C and 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 = (organic C extracted from fumigated soils) - (organic C extracted from non-fumigated soils) and k_{EC} = 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_{EC}, where E_N = (total N extracted from fumigated soils) - (total N extracted from non-fumigated soils) and k_{EN} = 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.

4.3.4 Ergosterol and cellulase activity

Ergosterol was measured 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 rev min^{-1} (Djajakirana et al. 1996). Ergosterol was determined by reversed-phase HPLC with 100% methanol as the mobile phase and a resolution of detection 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 g of moist soil was incubated with 5 ml acetate buffer and 0.5 g avicel for 16 h at 40°C. After centrifugation (2500 *g,* 10 min), reducing sugars in the supernatants were estimated by the method of Nelson and Somogyi (Spiro 1966).

4.3.5 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. All statistical analyses were performed using Stat View 5.0 (SAS Inst. Inc.).

Table 1. Cumulative CO_2 evolution over the 42-day incubation at 30ºC; 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	ΣCO_2-C ($\mu g\ g^{-1}$ soil)	0.5 M K_2SO_4 extractable C ($\mu g\ g^{-1}$ soil)		Ergosterol ($\mu g\ g^{-1}$ 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
0.5%F	1120 c	134 c	40 a	0.51 c	0.18 c	0.28 a	0.08 b
0.5%F+0.25%G	1630 b	879 b	40 a	0.76 b	0.23 b	0.15 b	0.09 ab
0.5%F+0.5%G	2320 a	1624 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 addition; %G = glucose addition; CV = mean coefficient of variation between replicate measurements; Different letters indicate a significant difference ($P < 0.05$, Scheffé, n = 4).

4.4 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 in the pure filter cake treatment (0.5%F) to 41% in the 0.5%filter cake +0.25% glucose treatment (0.5%F+0.25%G) to 48% in the 0.5% filter cake +0.5% glucose treatment (0.5%F+0.5%G).

The content of K_2SO_4 extractable C decreased in the control treatment by 35% during the 42-day incubation (Table 1). In the organic amendment treatments, 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 0.5%F+0.25%G and 0.5%F+0.5%G. 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 treatment from 27 µg N g^{-1} soil (0.5%F), to 82 µg N g^{-1} soil (0.5%F+0.25%G) and to 135 µg N g^{-1} soil (0.5%F+0.5%G). 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 d^{-1} in the sole filter cake treatment and at a rate of 1.5 µg N g^{-1} soil d^{-1} in the two glucose treatments. In the control treatment, the N mineralization rate was constantly at 0.64 µg N g^{-1} soil d^{-1} throughout the experiment.

Microbial biomass C remained constantly 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). In the pure filter cake treatment, 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 pass the initial value (Fig. 2a). In the two glucose treatments, the maximum microbial biomass C contents were measured at day 0, exceeding 4 and 6 times the microbial biomass C of the control soil. Then, the microbial biomass C content declined until the end of the experiment, reaching contents below 50% of the maximum content. In the pure filter cake treatment, the 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 0.5%F+0.25%G, microbial biomass N was constant at 22 µg g^{-1} soil throughout the incubation, but in treatment

0.5%F+0.5%G 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 treatment (Fig. 2d).

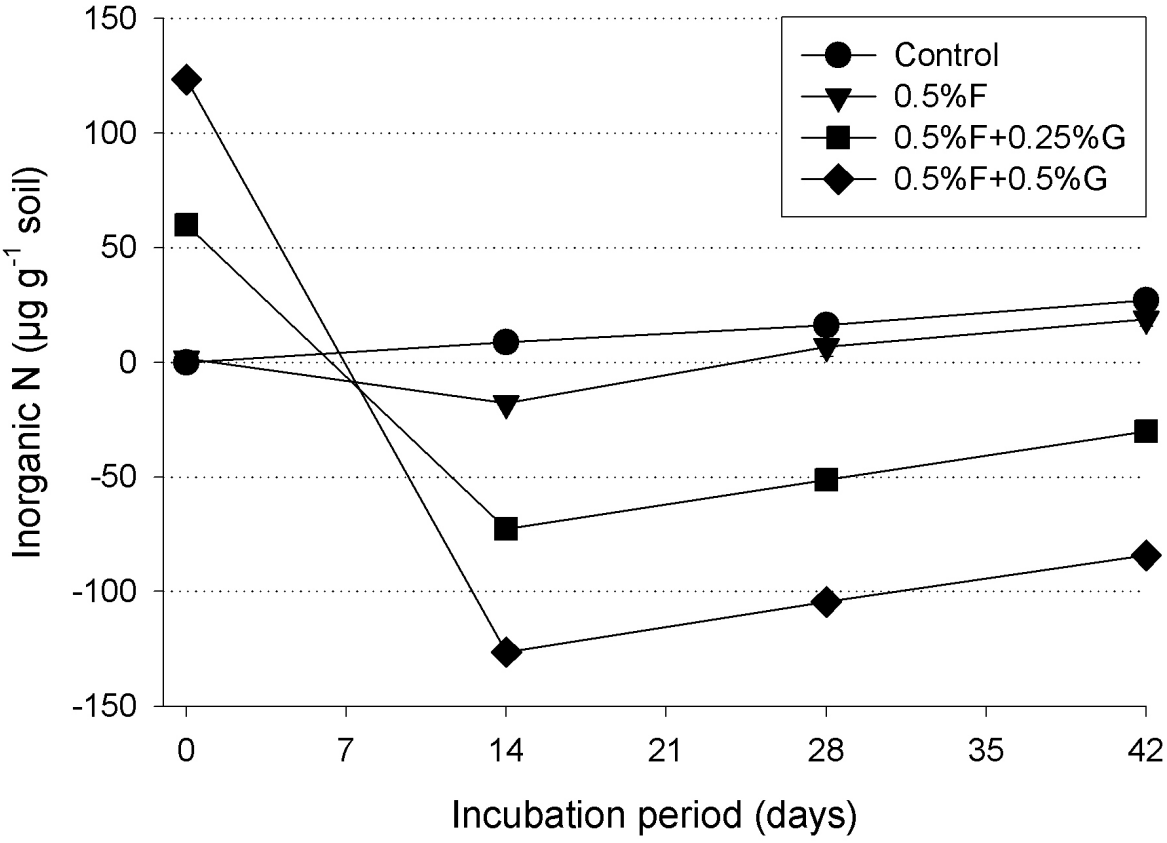

Fig. 1. Changes in the content of 0.5 M K_2SO_4 extractable inorganic N (NO_3-N + NH_4-N) during the 42-day incubation at 30ºC; %F = sugarcane filter cake addition; %G = glucose addition; one standard deviation is smaller than the symbols in all cases.

The organic amendments always led to an immediate 3- to 8-fold increase in ergosterol within 6 h after addition, compared with the control soil (Table 1). The increase in ergosterol was stronger than that of microbial biomass C, leading to increased ergosterol-to-microbial biomass C ratios, especially with increasing glucose concentrations. Only in the control treatment did the ergosterol-to-microbial biomass C ratio decline slightly until the end of the incubation, but that of the amendment treatments was always below that of the control treatment, in most cases significantly. The addition of

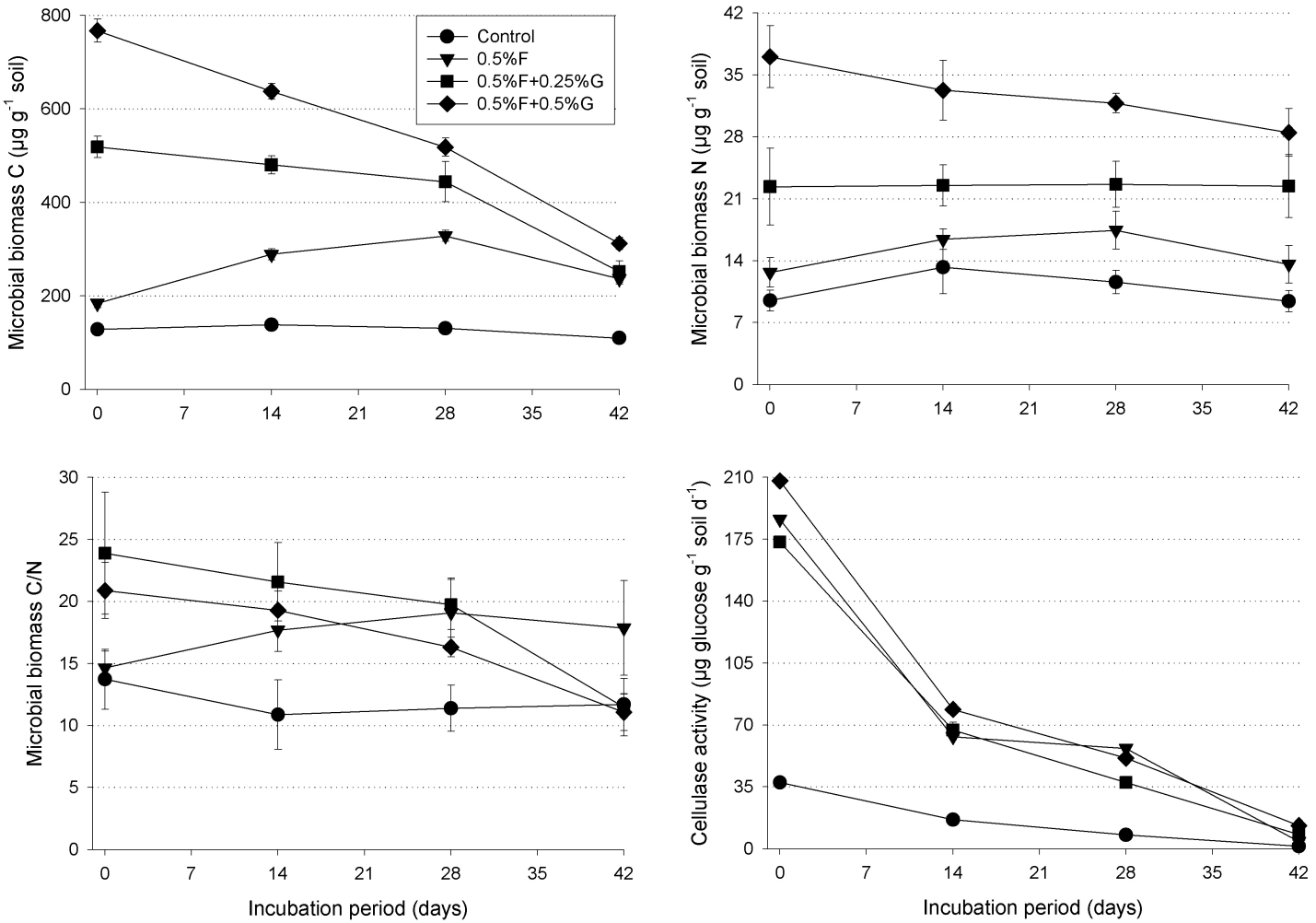

Fig. 2. Changes in (a) the contents of microbial biomass C, (b) the contents of microbial biomass N, (c) the microbial biomass C/N ratio and (d) cellulase activity during the 42-day incubation at 30ºC; %F = sugarcane filter cake addition; %G = glucose addition; bars indicate ± one standard deviation, but they are not always larger than the symbols.

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.

4.5 Discussion

4.5.1 Microbial activity indices

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 earlier reported for acidic (Rasul et al. 2006) and for alkaline soils (Khan et al. 2008) at different degrees of salinity. Assuming that glucose addition affected neither the decomposition of autochthonous soil organic matter nor that of sugar cane 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 sugar cane 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) and Pathak and Rao (1998). 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 alkaline soil used in the present study in accordance with Luna-Guido et al. (2000).

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. 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 day 14 to 42, the N re-mineralization rate from these freshly formed microbial residues was similar in the pure sugarcane filter cake treatment 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 in all three amendment treatments 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.

4.5.2 Microbial biomass indices

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 colonised 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 in the amendment treatments 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% (0.5%F+0.25%G) and 115% (0.5%F+0.5%G) of the loss in glucose C after 6 h, respectively. In contrast, the net-increase in microbial biomass N accounted for only 50% (0.5%F+0.25%G) and 66% (0.5%F+0.5%G) 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 metabolisation. 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.

The increase in ergosterol content in the two glucose treatments during the 6 h after amendment indicates that a certain percentage must be immediately metabolized by fast growing fungi, such as yeasts. 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 of the pure sugarcane filter cake treatment 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, 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.

4.6 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 immobilisation 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 in all three amendment treatments from day 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.

4.7 Acknowledgements

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4.8 References

Alef K, Nannipieri P (1995) Cellulase activity. In: Alef K, Nannipieri P (eds) Methods in applied soil microbiology and biochemistry. Academic Press, London, pp 345-349

Anderson TH, Gray TRG (1990) Soil microbial carbon uptake characteristics in relation to soil management. FEMS Microbiol Lett 74:11-19

Badole SB, More SD, Raut MM (2001) Residual effect of different organic and inorganic nutrient sources on yield and quality of groundnut. Ann. Plant Physiol. 15:54-57

Brookes PC, Landman A, Pruden G, Jenkinson DS (1985) Chloroform fumigation and the release of soil nitrogen: A rapid direct extraction method for measuring microbial biomass nitrogen in soil. Soil Biol Biochem 17:837-842

Chander K, Joergensen RG (2001) Decomposition of ^{14}C glucose in two soils with different levels of heavy metal contamination. Soil Biol Biochem 33:1811-1816

Chander K, Joergensen RG (2007) Microbial biomass and activity indices after organic substrate addition to a selenium contaminated soil. Biol Fertil Soils 44:241-244

Conde E, Cardenas M, Ponce-Mendoza A, Luna-Guido ML, Cruz-Mondragon C, Dendooven L (2005) The impacts of inorganic nitrogen application on mineralization of ^{14}C-labelled maize and glucose, and on priming effect in saline alkaline soil. Soil Biol Biochem 37:681-691

Coody PN, Sommers LE, Nelson DW (1986) Kinetics of glucose uptake by soil microorganisms. Soil Biol Biochem 18:283-289

Darrah PR, Nye PH, White RE (1987) The effect of high solute concentrations on nitrification rates in soil. Plant Soil 97:37-45

Dee BM, Haynes RJ, Graham MH (2003) Changes in soil acidity and the size and activity of the microbial biomass in response to the addition of sugar mill wastes. Biol Fertil Soils 37:47-54

Dilly O, Nannipieri P (2001) Response of ATP content, respiration rate and enzyme activities in an arable and a forest soil to nutrient additions. Biol Fertil Soils 34:64-72

Djajakirana G, Joergensen RG, Meyer B (1996) Ergosterol and microbial biomass relationship in soil. Biol Fertil Soils 22:299-304

Falchini L, Naumova N, Kuikman PJ, Bloem J, Nannipieri P (2003) CO_2 evolution and denaturing gradient gel electrophoresis profiles of bacterial communities in soil following addition of low molecular weight substrates to simulate root exudation. Soil Biol Biochem 35:775-782

Formowitz B, Schulz MC, Buerkert A, Joergensen RG (2007) Reaction of microorganisms on rewetting in dry continuous cereals and legume rotation soils of semi-arid Sub-Saharan Africa. Soil Biol Biochem 39:1512-1517

Hamer U, Marschner B, Brodowski S, Amelung W (2004) Interactive priming of black carbon and glucose mineralisation. Org Geochem 35:823-830

Hill PW, Farrar JF, Jones DL (2008) Decoupling of microbial glucose uptake and mineralization in soil. Soil Biol Biochem 40:616-624

Joergensen RG, Mueller T (1996) The fumigation-extraction method to estimate soil microbial biomass: Calibration of the k_{EN} value. Soil Biol Biochem 28:33-37

Joergensen RG, Raubuch M (2002) Adenylate energy charge of a glucose-treated soil without adding a nitrogen source. Soil Biol Biochem 34:1317-1324

Jones DL, Murphy DV (2007) Microbial response time to sugar and amino acid additions to soil. Soil Biol Biochem 39:2178-2182

Kautz T, Wirth S, Ellmer F (2004) Microbial activity in a sandy arable soil is governed by the fertilization regime. Eur J Soil Biol 40:87-94

Khan KS, Gattinger A, Buegger F, Schloter M, Joergensen RG (2008) Microbial use of organic amendments in saline soils monitored by changes in the $^{13}C/^{12}C$ ratio. Soil Biol Biochem (doi:10.1016/j.soilbio.2007.12.016)

Kuzyakov Y, Friedel JK, Stahr K (2000) Review of mechanisms and quantification of priming effects. Soil Biol Biochem 32:1485-1498

Kuzyakov Y, Hill PW, Jones DL (2007) Root exudate components change litter decomposition in a simulated rhizosphere depending on temperature. Plant Soil 290:293-305

Linkins AE, Sinsabaugh RL, McClaugherty CA, Melills JM (1990) Cellulase activity on decomposing leaf litter in microcosms. Plant Soil 123:17-25

Luna-Guido ML, Dendooven L (2001) Simulating the dynamics of glucose and NH_4^+ in alkaline saline soils of the former Lake Texcoco with the Detran model. Eur J Soil Sci 52:269-277

Luna-Guido ML, Beltran-Hernandez RI, Solis-Ceballos NA, Hernandez-Chavez N, Mercado-Garcia F, Olalde-Portugal V, Catt JA, Dendooven L (2000) Chemical and biological characteristics of alkaline saline soils from the former Lake Texcoco as affected by artificial drainage. Biol Fertil Soils 32:102-108

Mayer J, Buegger F, Jensen ES, Schloter M., Heß J (2004) Turnover of grain legume N rhizodeposits and effect of rhizodeposition on the turnover of crop residues. Biol Fertil Soils 39:153–164

Muhammad S, Müller T, Joergensen RG (2006) Decomposition of pea and maize straw in Pakistani soils along a gradient in salinity. Biol Fertil Soils 43:93-101

Muhammad S, Müller T, Joergensen RG (2008) Relationships between soil biological and other soil properties in saline and alkaline arable soils from the Pakistani Punjab. J Arid Environ (doi:10.1016/j.jaridenv.2007.06.016)

Nannipieri P, Muccini L, Ciardi C (1983) Microbial biomass and enzyme activities: Production and persistence. Soil Biol Biochem 15:679-685

De Nobili M, Contin M, Mondini C, Brookes PC (2001) Soil microbial biomass is triggered into activity by trace amounts of substrate. Soil Biol Biochem 33:1163-1170

De Nobili M, Contin M, Brookes PC (2006) Microbial biomass dynamics in recently air-dried and rewetted soils compared to others stored air-dry for up to 103 years. Soil Biol Biochem 38:2871-2881

Oren A (1999) Bioenergetic aspects of halophilism. Microbiol Mol Biol Rev 63:334-348

Parthasarathi K, Ranganathan LS (2000) Aging effect on enzyme activities in pressmud vermicasts of *Lampito mauritii* (Kinberg) and *Eudrilus eugeniae* (Kinberg). Biol Fertil Soils 30:347-350

Pathak H, Rao DLN (1998) Carbon and nitrogen mineralization from added organic matter in saline and alkali soils. Soil Biol Biochem 30:695-702

Pavel R, Doyle J, Steinberger Y (2004) Seasonal patterns of cellulase concentration in desert soil. Soil Biol Biochem 36:49-554

Powlson DS, Hirsch PR, Brookes PC (2001) The role of soil microorganisms in soil organic matter conservation in the tropics. Nutr Cycl Agroecosys 61:41-51

Rasul G, Appuhn A, Müller T, Joergensen RG (2006) Salinity induced changes in the microbial use of sugar cane filter cake material under acidic soil conditions. Appl Soil Ecol 31:1-10

Spiro RG (1966) Analysis of sugars found in glycoproteins. In: Neufeld EF, Ginsberg V (eds.) Methods in enzymology, vol. 8. Academic Press, London, pp 326

Vance ED, Brookes PC, Jenkinson DS (1987) An extraction method for measuring soil microbial biomass C. Soil Biol Biochem 19:703-707

Vinten AJA, Whitmore AP, Bloem J, Howard R, Wright F (2002) Factors affecting immobilisation/mineralisation kinetics for cellulose-, glucose-, straw-amended sandy soils. Biol Fertil Soils 36:190-199

Wu J, Joergensen RG, Pommerening B, Chaussod R, Brookes PC (1990): Measurement of soil microbial biomass-C by fumigation-extraction - an automated procedure. Soil Biol Biochem 22:1167-1169

Wu J, Brookes PC, Jenkinson DS (1993) Formation and destruction of microbial biomass during the decomposition of glucose and ryegrass in soil. Soil Biol Biochem 25:1435-1441

Yadvinder-Singh, Bijay-Singh, Gupta RK, Ladha JK, Bains JS, Singh J (2008) Evaluation of press mud cake as a source of nitrogen and phosphorus for rice–wheat cropping system in the Indo-Gangetic plains of India. Biol Fertil Soils (DOI 10.1007/s00374-007-0258-y)

Chapter 5 – Decomposition of organic amendments to an artificial saline soil varying in anion composition and inoculum at different temperatures

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5.1 Abstract

An incubation experiment was carried out with the objective of assessing the effects of salt additions containing different anions (Cl^-, SO_4^{2-}, HCO_3^-) on the microbial use of sugarcane filter cake and dhancha leaves amended to inoculated sterile quartz sand. In a subsequent experiment, the objective was to assess the effects of inoculum and temperature on the decomposition of sugar cane filter cake. In experiment 1, sugarcane filter cake led to significantly lower respiration rates, lower contents of extractable C and N, and lower contents of microbial biomass C and N than dhancha leaves, but to a higher respiratory quotient RQ and to a higher content of the fungal biomarker ergosterol. The RQ was significantly increased after salt addition, when comparing the average of all salinity treatments with the control. Differences in anion composition had no clear effects on the RQ values. In experiment 2, the rise in temperature from 20 to 40°C increased the CO_2 production rate by a factor of 1.6, the O_2 consumption rate by a factor of 1.9 and the ergosterol content by 60%. In contrast, the contents of microbial biomass N decreased by 60% and the RQ by 13%. The effects of the inoculation with a saline soil were in most cases negative and did not indicate a better adaptation of these organisms to salinity. The general effects of anion composition on microbial biomass and activity indices were small and inconsistent. Only the fraction of K_2SO_4 extractable C and N in non-fumigated soil was consistently increased in the 1.2 M $NaHCO_3$ treatment of both experiments. In contrast to the small salinity effects, the quality of the substrate has overwhelming effects on microbial biomass and activity indices, especially on the fungal part of the microbial community.

Keywords Sugarcane filter cake • Dhancha • CO_2 production • O_2 consumption • Ergosterol • Chloride • Sulfate • Carbonate

5.2 Introduction

Decomposition of organic substrates and the release of nutrient elements are key functions of soil microorganisms (Swift et al. 1979). Salinisation is an increasing threat to agricultural soils throughout the world (Keren 2000; Qadir et al. 2000) and thus also to microorganisms in these soils (Zahran 1997). For this reason, salinity effects on the microbial decomposition of organic amendments and soil microbial processes have received increasing interest in the last decade (Rietz and Haynes 2003; Mamilov et al. 2004; Yuan et al. 2007). Salinisation has reduced preferentially the fungal part of the soil microbial community in alkaline sandy loams in South Australia (Pankhurst et al. 2001), but also in acidic sandy loams in Germany (Sardinha et al. 2003). The specific reduction of fungi may reduce the decomposition of complex organic material in saline soils (Badran 1994), because this group of organisms is especially important for the breakdown of lignin and cellulose, important components of plant residues (Swift et al. 1979, Harper and Lynch 1985). However, the effects of salinity on the decomposition of organic amendments are contradictory. Decreases in C mineralization have been reported with increasing salinity (Laura 1974; Nelson et al. 1996; Pathak and Rao 1998; Luna-Guido et al. 2003; Wichern et al. 2006), but also no effects and even increases (Li et al. 2006a; Rasul et al. 2006).

A large variety of responses by different microbial communities to the same substrate is a common feature in laboratory decomposition experiments (Harper and Lynch 1985; Nicolardot et al. 1994; Potthoff et al. 2001) and might be simply an expression of real differences between soils, but could also be an expression of methodological constraints. However, investigations of salinity effects are biased by several methodological problems. If natural soil gradients are analyzed, differences in the levels of soil organic matter and, as a consequence, also that of soil microbial biomass may mask or amplify salinity effects (Rasul et al. 2006, Tripathi et al. 2006). A larger microbial biomass results in a larger incorporation of substrate into the microbial biomass. If easily decomposable substrates are added, a high microbial biomass soil often leads to a significantly lower C mineralization rate than a low microbial biomass C soil (Witter and Kanal 1998; Chander and Joergensen 2001). If a more recalcitrant material is added, a high microbial biomass soil usually leads to a higher C mineralization rate than a low microbial biomass C soil (Khan and Joergensen 2006).

If soils are investigated after artificial salinisation, the soil microorganisms are not adapted to this type of stress (Laura et al. 1974; Nelson et al. 1996; Wichern et al. 2006). In this type of experiment, NaCl is usually used to create salinity. However, the salt of natural saline soils is a mixture of chlorides, sulfates and carbonates. Salts from different saline soils of the Pakistani Punjab contained 20% chlorides, 30% sulfates and 50% carbonates (Muhammad et al. 2008). These anions behave differently in soil, because SO_4^{2-} has a larger hydration diameter than Cl^- and a stronger tendency to form ion pairs in soil solution (Bohn et al. 1985; Li et al. 2006). Consequently, the different anions may have different effects on soil microorganisms. For example, SO_4^{2-} salts were less toxic to nitrification than Cl^- salts (Sindhu and Cornfield 1967).

In the present study, sterile sand was used that did not contain any soil organic matter, i.e. the experiments were not biased by differences in this important soil property. The sterile sand was inoculated with a saline soil with the objective of assessing the effects of salt additions containing different anions (Cl^-, SO_4^{2-}, HCO_3^-) on the microbial use of sugarcane (*Saccharum sp*.) filter cake and dhancha (*Sesbania bispinosa* (Jacq.) W. Wight) leaves. Both organic amendments have similar C/N ratios, but different organic matter composition. In a subsequent experiment, the objective was to assess the effects of inoculum (saline and non-saline soil) and temperature (20 and 40ºC) on the decomposition of sugar cane filter cake using a similar experimental set-up.

5.3 Materials and Methods

5.3.1 Substrate, inoculums and amendments

The dhancha (*Sesbania bispinosa* (Jacq.) W. Wight) leaves were taken from a field experiment at Rawalpindi, Pakistan. The leaves were air-dried, milled (< 0.5 mm) and transported to Germany. 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 (Rasul et al. 2006). The elemental composition of sugarcane filter cake and dhancha leaves is shown in Table 1. The soil described by Rasul et al. (2008a) was used as a saline inoculum and the soil described by Rasul et al. (2008b) as a non-saline inoculum.

Table 1 Concentrations of elements and elemental ratios in dhancha leaves and sugarcane filter cake (in brackets: ± standard deviation)

	Filter cake	Dhancha
Total C (mg g^{-1})	420 (8.0)	476 (2.7)
Total N (mg g^{-1} soil)	34 (1.4)	37 (1.9)
Total P (mg g^{-})	12.3 (0.3)	1.9 (0.1)
Total S (mg g^{-1})	3.9 (0.1)	2.8 (0.1)
K (mg g^{-1})	11.7 (0.3)	9.6 (0.2)
Mg (mg g^{-1})	3.9 (0.1)	3.1 (0.1)
Ca (mg g^{-1})	23.4 (0.5)	35.6 (1.0)
Total C/N	12.4	12.7
Total C/P	34	253
Total C/S	108	168

5.3.2 Incubation procedure

Experiment 1 was carried out with two amendments: (A) 10 mg C g^{-1} sugarcane filter cake and (B) 10 mg g^{-1} dhancha leaves. Each of the two amendments was subjected to the following eight salinity treatments: (1) non-saline control, (2) +0.4 mM NaCl, (3) +1.2 mM NaCl, (4) 0.2 mM Na_2SO_4, (5) 0.6 mM Na_2SO_4, (6) 0.4 mM $NaHCO_3$, (7) 1.2 mM $NaHCO_3$, and (8) 0.4 mM NaCl + 0.2 mM Na_2SO_4 + 0.4 mM $NaHCO_3$. The experiment was repeated four times with each amendment and salinity treatment for 6 days at 30ºC in the dark using a 500 l incubator.

Experiment 2 was carried out with 10 mg C g^{-1} s sugarcane filter cake and two different inoculums: (A) saline soil and (B) non-saline soil. Each of the two inoculum treatments was subjected to the following 5 salinity treatments: (1) non-saline control, (2) +1.2 mM NaCl, (3) 0.6 mM Na_2SO_4, (4) 1.2 mM $NaHCO_3$, and (5) 0.4 mM NaCl + 0.2 mM Na_2SO_4 + 0.4 mM $NaHCO_3$. The experiment was repeated four times with each amendment and salinity treatment for 6 days at (a) 20ºC and (b) 40ºC in the dark using a 500 l incubator.

For each treatment of the two experiments, 49 g (on an oven-dry basis) of sterile quartz sand was thoroughly mixed with 1 g (on an oven-dry basis) soil as inoculum and

the different organic amendments. These mixtures were weighed into 80 ml incubation cylinders made of stainless steel nets and adjusted to 50% water holding capacity with water or the different salt solutions. The cylinders were placed into 500 ml incubation vessels containing 20 ml 1 M NaOH at the bottom. The CO_2 evolved was determined by back-titration to pH 8.3 of the excess NaOH with 1 M HCl after addition of 0.5 M $BaCl_2$ solution. The O_2 consumed was measured at the same time using an Aqualytic (Darmstadt, Germany) tension-recording device (Robertz et al. 1999).

5.3.3 Analytical procedures

The fungal cell-membrane component ergosterol was extracted from 1 g of moist samples with 100 ml ethanol (Djajakirana et al. 1996). Then, ergosterol was determined by reversed-phase HPLC with 100% methanol as the mobile phase and detected at a wavelength of 282 nm. Microbial biomass C and biomass N were estimated by fumigation-extraction (Brookes et al. 1985; Vance et al. 1987). Two portions of 15 g moist substrate were taken from the 50 g sample used for the incubation. One portion was fumigated for 24 h at 25°C with ethanol-free $CHCl_3$. Following fumigant removal, the samples were extracted with 100 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 = (organic C extracted from fumigated soils) - (organic C extracted from non-fumigated soils) and k_{EC} = 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 = (total N extracted from fumigated soils) - (total N extracted from non-fumigated soils) and k_{EN} = 0.54 (Brookes et al. 1985; Joergensen and Mueller 1996). In the 0.5 M K_2SO_4 extracts of non-fumigated samples, NH_4-N and NO_3-N was additionally measured using segmented continuous flow analysis followed by spectrometric detection. However, inorganic N concentrations never exceeded those of the blank solutions.

5.3.4 Statistical analysis

The results presented in the tables are arithmetic means and expressed on an oven-dry basis (about 24 h at 105°C). The significance of effects in experiment I was tested by a

two-way ANOVA with amendments and salinity as independent factors. The significance of effects in experiment II was tested by a three-way ANOVA with salinity, inoculum and temperature as independent factors. The factor salinity comprised all 7 treatments with salt addition in experiment 1 and all 4 treatments with salt addition in experiment 2. The significance of salt-specific effects was analyzed by means of a one-way ANOVA using Fisher's PLSD (protected least significant difference) post-hoc test. All data were ln-transformed for statistical evaluation, except the ergosterol content in experiment 2. All statistical analyses were performed using StatView 5.0 (SAS Inst. Inc.).

5.4 Results

5.4.1 Differences between the organic amendments

In experiment 1, sugarcane filter cake led to significantly lower respiration rates than dhancha leaves (Table 2). The CO_2 production rate was on average 64% lower and the O_2 consumption rate 70% lower. For this reason, the mean respiratory quotient (RQ) was a significant 14% higher after filter cake addition in comparison with dhancha leaves. The RQ was significantly higher after salt addition, comparing the average of the salinity treatments with the control. Differences in anion composition had no clear effects on the RQ values.

Sugarcane filter cake led to 75% lower mean concentrations of 0.5 M K_2SO_4 extractable C and N in comparison with dhancha leaves (Table 3). The extractable components did not show clear overall salinity effects. However, the amendment-specific one-way ANOVA revealed a significant increase of extractable C in the 1.2 M $NaHCO_3$ treatment of both amendments in comparison with the control treatment. In the 1.2 M $NaHCO_3$ treatment of the filter cake amendment, the content of extractable N was also significantly increased. In contrast to the two $NaHCO_3$ treatments, the two NaCl and the two Na_2SO_4 treatments resulted in significantly lower concentrations of extractable C and N in comparison with the control treatment.

The microbial biomass C contents were on average 75% lower in the filter cake than in the dhancha treatments. In contrast, the contents of microbial biomass N were identical in both amendment treatments. Also microbial biomass C and N did not show clear overall salinity effects. However, the amendment-specific one-way ANOVA revealed a significantly lower microbial biomass C content in the 1.2 M $NaHCO_3$

treatment of the dhancha amendments in comparison with the control treatment and significantly higher microbial biomass N contents in the two NaCl and the two Na_2SO_4 treatments. The ergosterol content in the filter cake was more than twice that in the dhancha amendments and the ergosterol-to-microbial biomass C ratio was nearly eight times larger (Table 2). Also the ergosterol content did not show clear overall salinity effects. However, the amendment-specific one-way ANOVA revealed a significantly higher ergosterol content in the 1.2 M $NaHCO_3$ treatment of the dhancha amendments in comparison with the control treatment. In contrast, the different anion compositions and salt concentrations led to a complex picture of decreases and increases.

5.4.2 Differences in temperature

In experiment 2, the rise in temperature from 20 to 40°C increased the CO_2 production rate by a factor of 1.6 and the O_2 consumption rate by a factor of 1.9 (Table 4). The salinity treatments slightly increased the mean CO_2 production rate, especially due to the 1.2 M $NaHCO_3$ treatment, but the O_2 consumption rate was not significantly affected. There was a strong tendency for the O_2 consumption rate to be lower in the salinity treatments.

The rise in temperature also led to a significant increase in the contents of 0.5 K_2SO_4 extractable N (+40%) (Table 5) as well as ergosterol (+60%) and in the ergosterol-to-microbial biomass C ratio (+80%) (Table 4). In contrast, the content of microbial biomass N (-60%) (Table 5) and the RQ (-13%) (Table 4) decreased significantly. The salinity treatments did not show any clear overall effects on the extractable components and the three microbial biomass indices. However, the amendment-specific one-way ANOVA revealed a significant 2-fold higher content of extractable C in the 1.2 M $NaHCO_3$ treatment in comparison with the control treatment. The contents of microbial biomass C, biomass N and ergosterol, but especially the ergosterol-to-microbial biomass C ratio showed significant interactions between temperature and salinity, i.e. a disproportionate increase in ergosterol content in the salinity treatments at higher temperatures combined with an disproportionate decrease in microbial biomass.

Table 2 Salt treatment-specific and main effects of amendments on CO_2 production, O_2 consumption, respiratory quotient RQ, ergosterol content, and the ergosterol-to-microbial biomass C ratio at the end of a 6-d incubation period at 30ºC; the factor salinity comprised all 7 treatments with salt addition

Treatment	CO_2-C ($\mu g\ g^{-1}\ h^{-1}$)	O_2 ($\mu g\ g^{-1}\ h^{-1}$)	RQ CO_2/O_2 ($mol\ mol^{-1}$)	Ergosterol ($\mu g\ g^{-1}$)	Ergosterol/ microbial biomass C (%)
Dhancha					
Control	16.4 ab	58 a	0.75 a	0.87 b	0.14 bc
0.4 mM NaCl	17.0 a	54 a	0.84 a	0.92 b	0.14 bc
1.2 mM NaCl	17.1 a	54 a	0.85 a	1.24 b	0.12 bc
0.2 mM Na_2SO_4	16.0 b	57 a	0.75 a	0.87 b	0.11 bc
0.6 mM Na_2SO_4	15.9 b	56 a	0.76 a	0.75 b	0.06 c
0.4 mM $NaHCO_3$	16.3 ab	57 a	0.77 a	0.87 b	0.14 b
1.2 mM $NaHCO_3$	16.8 a	58 a	0.78 a	1.93 a	0.42 a
1.0 mM mixture	16.6 ab	55 a	0.81 a	1.16 b	0.12 bc
Filter cake					
Control	5.4 b	18 a	0.78 c	2.12 cd	1.04 ab
0.4 mM NaCl	5.6 b	16 a	0.94 ab	1.78 d	1.10 ab
1.2 mM NaCl	5.2 b	16 a	0.88 b	2.76 bc	1.92 a
0.2 mM Na_2SO_4	5.3 b	17 a	0.84 bc	3.01 ab	1.60 a
0.6 mM Na_2SO_4	5.6 b	19 a	0.80 c	1.85 d	0.80 b
0.4 mM $NaHCO_3$	6.2 ab	18 a	0.92 ab	2.54 bc	1.35 ab
1.2 mM $NaHCO_3$	7.4 a	19 a	1.03 a	2.08 cd	0.73 b
1.2 mM mixture	6.0 ab	17 a	0.97 a	3.35 a	1.49 a
Dhancha	16.5	56	0.79	1.08	0.16
Filter cake	5.8	17	0.90	2.44	1.25
Probability levels (degree of freedom)					
Amendment (1)	<0.01	<0.01	0.03	<0.01	<0.01
Salinity (1)	0.32	0.29	0.01	0.32	0.77
S x A	0.40	0.85	0.18	0.86	0.83
CV (± %)	9.4	12	7.6	25	43

CV = mean coefficient of variation between replicate samples; different letters within an amendment-specific column indicate a significant difference (PLSD-test, $P < 0.05$, n = 4).

Table 3 Treatment-specific and main effects of amendments on extractable organic matter and microbial biomass at the end of a 6-d incubation period at 30ºC; the factor salinity comprised all 7 treatments with salt addition

	0.5 M K_2SO_4 extractable		Microbial biomass	
	C (µg g^{-1})	N (µg g^{-1})	C (µg g^{-1})	N (µg g^{-1})
Dhancha				
Control	1000 b	190 a	790 b	19 c
0.4 mM NaCl	580 de	130 c	870 b	40 ab
1.2 mM NaCl	510 e	120 c	1050 ab	49 a
0.2 mM Na_2SO_4	650 d	140 bc	920 b	43 ab
0.6 mM Na_2SO_4	730 cd	160 bc	1270 a	35 b
0.4 mM $NaHCO_3$	1090 ab	190 a	610 b	20 c
1.2 mM $NaHCO_3$	1260 a	170 ab	460 c	15 c
1.2 mM mixture	830 c	150 bc	1120 ab	38 ab
Filter cake				
Control	240 b	27 bc	220 a	32 ab
0.4 mM NaCl	200 b	23 c	180 a	24 b
1.2 mM NaCl	250 b	29 bc	200 a	30 ab
0.2 mM Na_2SO_4	240 b	28 bc	240 a	36 ab
0.6 mM Na_2SO_4	210 b	24 c	230 a	34 ab
0.4 mM $NaHCO_3$	240 b	28 bc	230 a	30 ab
1.2 mM $NaHCO_3$	430 a	52 a	290 a	44 a
1.2 mM mixture	280 b	34 b	250 a	38 ab
Dhancha	830	160	890	32
Filter cake	260	30	230	34
Probability levels (degree of freedom)				
Amendment (1)	<0.01	<0.01	<0.01	0.18
Salinity (1)	0.42	0.50	0.68	0.13
S x A	0.17	0.13	0.65	0.18
CV (± %)	15	11	29	33

CV = mean coefficient of variation between replicate samples; different letters within an amendment-specific column indicate a significant difference (PLSD-test, $P < 0.05$, n = 4).

Table 4 Main effects of salt treatments, inoculum and temperature on CO_2 production, O_2 consumption, respiratory quotient RQ, ergosterol content, and the ergosterol-to-microbial biomass C ratio at the end of a 6-d incubation period with sugarcane filter cake; the factor salinity comprised all 4 treatments with salt addition

Treatment	CO_2-C ($\mu g\ g^{-1}\ h^{-1}$)	O_2 ($\mu g\ g^{-1}\ h^{-1}$)	RQ CO_2/O_2 ($mol\ mol^{-1}$)	Ergosterol ($\mu g\ g^{-1}$)	Ergosterol/ microbial biomass C (%)
Control	6.2 a	23 a	0.75 b	1.8 ab	1.6 ab
1.2 mM NaCl	5.9 a	21 a	0.79 b	1.5 b	1.0 b
0.6 mM Na_2SO_4	6.2 a	22 a	0.77 b	1.7 ab	1.1 ab
1.2 mM $NaHCO_3$	7.8 b	22 a	0.99 a	2.1 a	1.7 a
1.0 mM mixture	6.8 ab	23 a	0.83 a	2.1 a	1.7 a
Non-saline inoculum	6.7	23	0.80	1.7	1.5
Saline inoculum	6.5	21	0.85	2.0	1.4
20ºC	5.0	15	0.89	1.4	1.0
40ºC	8.1	29	0.77	2.3	1.8
Probability levels (degree of freedom)					
Salinity (1)	0.03	0.07	0.01	1.00	0.47
Inoculum (1)	0.67	<0.01	0.01	0.49	0.04
Temperature (1)	<0.01	<0.01	<0.01	<0.01	<0.01
S x I	0.19	0.08	0.03	0.10	<0.01
S x T	0.34	0.72	0.57	0.04	<0.01
I x T	0.63	<0.01	<0.01	0.95	0.12
CV (± %)	3.0	5.5	6.0	21	33

CV = mean coefficient of variation between replicate samples; different letters within a column indicate a significant difference (PLSD-test, $P < 0.05$, n = 4).

Table 5 Main effects of salt treatments, inoculum and temperature on extractable organic matter and microbial biomass at the end of a 6-d incubation period with sugarcane filter cake; the factor salinity comprised all 4 treatments with salt addition

	0.5 M K_2SO_4 extractable		Microbial biomass	
	C (µg g^{-1})	N (µg g^{-1})	C (µg g^{-1})	N (µg g^{-1})
Control	280 b	32 b	163 a	25 a
1.2 mM NaCl	270 b	30 b	174 a	29 a
0.6 mM Na_2SO_4	250 b	29 b	171 a	30 a
1.2 mM $NaHCO_3$	620 a	72 a	143 a	21 a
1.0 mM mixture	310 b	35 b	133 a	20 a
Non-saline inoculum	360	42	146	25
Saline inoculum	330	38	167	26
20ºC	300	33	179	36
40ºC	390	47	134	14
Probability levels (degree of freedom)				
Salinity (1)	0.12	0.11	0.83	0.34
Inoculum (1)	0.72	0.84	<0.01	0.12
Temperature (1)	0.23	0.02	0.30	<0.01
S x I	0.49	0.50	0.01	0.72
S x T	0.24	0.69	0.01	0.04
I x T	0.79	0.83	0.01	0.05
CV (± %)	10	11	42	24

CV = mean coefficient of variation between replicate samples; different letters within a column indicate a significant difference (PLSD-test, $P < 0.05$, n = 4).

5.4.3 Differences in inoculum

In experiment 2, the inoculum of the filter cake / sterile sand mixture with a saline soil resulted in significantly lower O_2 consumption rates (Tables 4), but significantly higher RQ values (Table 4) and microbial biomass C contents (Table 5). Significant second

interactions on the RQ (Table 4) and microbial biomass C (Table 5) were caused by the considerably higher RQ values and microbial biomass C contents of the saline inoculum at 20°C in the salinity treatments.

5.5 Discussion

Sugarcane filter cake and dhancha leaves have very similar C and N contents. However, fresh dhancha leaves with regular polymeric structures are mineralized at markedly higher rates than the highly decomposed filter cake. The three microbial biomass C indices reacted quite differently to the substrate quality. Microbial biomass C reached considerably higher values with dhancha leaves than with filter cake amendments. The microbial biomass N contents did not differ between the two amendments and the ergosterol content was significantly higher in the filter cake amendments. This suggests that the decomposition of filter cake disproportionately promotes fungi in comparison with the leguminous dhancha leaves. A strong increase in ergosterol content was observed after filter cake amendment (Rasul et al. 2006) and a small increase in ergosterol after leguminous pea straw amendment (Muhammad et al. 2006). This would mean that a microbial biomass containing a higher percentage of bacteria exhibits a markedly larger microbial biomass C/N ratio than that with a higher percentage of fungi. This result contradicts the view repeatedly stated that high microbial biomass C/N ratios indicate fungal dominance (Joergensen et al. 1995; Kao et al. 2006; Sparrow et al. 2006). The reasons for the larger microbial biomass C/N ratios, often found in soils with P deficiency (Salamanca et al. 2006; Joergensen and Emmerling 2006), are not fully understood. However, the dhancha leaves contain sufficient amounts of P. Whether other nutrients also lead to high microbial biomass C/N ratios is completely unknown.

The temperature has strong effects on all microbial activity and biomass indices analyzed after filter cake amendment. The mean Q_{10} value for the CO_2 production rate was 1.3 over the range of 20 to 40°C during a 6-day incubation period and thus identical to that obtained by Raubuch et al. (2007) for the range of 15 to 25°C investigating the decomposition rate of maize residues during a 49-day incubation period. The Q_{10} value for the O_2 consumption rate was 1.4 and, thus, only slightly higher. Such low Q_{10} values are typical for recalcitrant organic materials containing only small labile pools (Kirschbaum 2006). This indicates the importance of substrate availability to soil

microorganisms for CO_2 production and O_2 consumption. The reasons for the much lower microbial biomass C, but especially microbial biomass N content after 6 days of incubation at 40°C in comparison with 20°C are not fully understood. A strong decline was observed by Joergensen et al. (1990) during an extended incubation at 35°C. The lower microbial biomass content at 40°C was not mirrored by that of ergosterol. This may mean that ergosterol did not disappear immediately after cell death as observed by Zhao et al. (2005). However, also methodological problems of the fumigation extraction method cannot be completely excluded. Salinity obviously reduced the adsorption of microorganisms on the sand or substrate particles, which might have led to losses of microorganisms during the extraction of the non-fumigated samples. This is indicated by the high contents of K_2SO_4 extractable C and N at 40°C. However, these relatively high values might also be caused by a strong mobilization of a recalcitrant fraction (Jones et al. 2004).

The effects of the inoculation with a saline soil were generally small although some significant differences were observed. These effects were in most cases negative and did not indicate a better adaptation of these organisms to salinity, contradicting the findings of Wichern et al. (2006). A minor effect of the inoculum was also observed by Chander et al. (2002), indicating that soil organisms are likely less important for early decomposition than the substrate colonizing microorganisms (Flessa et al. 2002).

The general effects of salinity on the decomposition of sugarcane filter cake and on the microbial biomass and activity indices were small. This is consistent with the previous experiments (Rasul et al. 2006, 2008b), but in contrast to the results of Li et al. (2006), who used roughly 5- to 10-times lower NaCl and Na_2SO_4 concentrations. The respiratory quotient RQ was the only index that reacted consistently to salinity. The RQ values are all in the range given by Dilly (2001). Khan and Joergensen (2006) observed a very constant mean RQ of 1.13 after nettle amendment over a 6-day incubation, suggesting that this material was also used by anaerobic microorganisms (Theenhaus et al. 1997; Dilly 2003). Theenhaus et al. (1997) measured a mean RQ value of 1.19 for basal respiration in forest soils, but only a mean of 0.72 in arable soils low in soil organic matter. A low value suggests that aliphatic organic components, amino acids or refractory components containing relatively little oxygen were predominantly mineralized (Dilly 2001). The absence of soil organic matter may be one reason for the relatively low RQ values in comparison with Khan and Joergensen (2006).

Also, the general effects of anion composition on microbial biomass and activity indices were small and inconsistent: Only K_2SO_4 extractable C and N in non-fumigated soil were consistently increased in the 1.2 M $NaHCO_3$ treatment of both experiments. Also the CO_2 production rate of the filter cake amendment was significantly increased in both experiments. Labilization of organic matter at high pH in the presence of sodium ions might be an important reason for the low organic matter levels in the sodic soils, with negative feedback mechanisms on soil microorganisms (Laura 1973, 1976; Nelson et al. 1996; Muhammad et al. 2008).

5.6 Conclusions

The absence of clear salinity effects on microbial biomass and activity indices of the present incubation experiment give further evidence that salinity effects on soil microorganisms observed repeatedly under field conditions are caused by indirect effects on plant growth and on the physical structure of the soil. In contrast to the small salinity effects, the quality of the substrate has overwhelming effects on microbial biomass and activity indices, especially on the fungal part of the microbial community.

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5.8 References

Badran RAM (1994) Cellulolytic activity of some cellulose-decomposing fungi in salinized soils. Acta Mycol 29:245-221

Bohn HL, McNeal BL, O'Connor GA (1985) Soil chemistry, 2nd. Wiley, New York

Brookes PC, Landman A, Pruden G, Jenkinson DS (1985) Chloroform fumigation and the release of soil nitrogen: A rapid direct extraction method for measuring microbial biomass nitrogen in soil. Soil Biol Biochem 17:837-842

Chander K, Joergensen RG (2001) Decomposition of ^{14}C glucose in two soils with different levels of heavy metal contamination. Soil Biol Biochem 33:1811-1816

Chander K, Eberhardt U, Joergensen RG, Klein T (2002) Decomposition of carbon-14-labelled wheat straw in repeatedly fumigated and non-fumigated soils with different amounts of heavy metal contamination. Biol Fertil Soils 35:86-91

Dilly O (2001) Microbial respiratory quotient during basal metabolism and after glucose amendment in soils and litter. Soil Biol Biochem 33:117-127

Dilly O (2003) Regulation of the respiratory quotient of soil microbiota by availability of nutrients. FEMS Microbiol Ecol 43:375-381

Djajakirana G, Joergensen RG, Meyer B (1996) Ergosterol and microbial biomass relationship in soil. Biol Fertil Soils 22:299-304

Flessa H, Potthoff M, Loftfield N (2002) Greenhouse estimates of CO_2 and N_2O emissions following surface application of grass mulch: importance of indigenous microflora of mulch. Soil Biol Biochem 34:875-879

Harper SHT, Lynch JM (1985) Colonization and decomposition of straw by fungi. Trans Br Mycol Soc 85:655-661

Joergensen RG, Emmerling C (2006) Human impact on soil organisms and on current measurement methods. J Plant Nutr Soil Sci 169:295-309

Joergensen RG, Mueller T (1996) The fumigation-extraction method to estimate soil microbial biomass: Calibration of the k_{EN} value. Soil Biol Biochem 28:33-37

Joergensen RG, Brookes PC, Jenkinson DS (1990) Survival of the soil microbial biomass at elevated temperatures. Soil Biol Biochem 22:1129-1136

Joergensen RG, Anderson TH, Wolters V (1995) Carbon and nitrogen relationships in the microbial biomass of soils in beech (*Fagus sylvatica* L.) forests. Biol Fertil Soils 19:141-147

Jones DL, Shannon D, Murphy DV, Farrar J (2004) Role of dissolved organic nitrogen (DON) in soil N cycling in grassland soils. Soil Biol Biochem 36:749-756

Kao PH, Huang CC, Hseu ZY (2006) Response of microbial activities to heavy metals in a neutral loamy soil treated with biosolid. Chemosphere 64:63-70

Keren R (2000) Salinity. In: Sumner ME (ed) Handbook of soil science. CRC Press, Boca Raton, pp G3-G25

Khan S, Joergensen RG (2006) Decomposition of heavy metal-contaminated nettles (*Urtica dioica* L.) in soils differently subjected to heavy metal pollution by river sediments. Chemosphere 65:981-987

Kirschbaum MUF (2006) The temperature dependence of organic-matter decomposition – still a topic of debate. Soil Biol Biochem 38:2510-2518

Laura RD (1973) Effects of sodium carbonate on carbon and nitrogen mineralisation of organic matter added to soil. Geoderma 9:15-26

Laura RD (1974) Effects of neutral salts on carbon and nitrogen mineralisation of organic matter in soil. Plant Soil 41:113–127

Laura RD (1976) Effects of alkali salts on carbon and nitrogen mineralization of organic matter in soil. Plant Soil 41:587–596

Li X, Li F, Bhupinderpal-Singh, Rengel Z (2006a) Decomposition of maize straw in saline soil. Biol Fertil Soils 42:366–370

Li X, Li F, Ma Q, Cui Z (2006) Interactions of NaCl and Na_2SO_4 on soil organic C mineralization after addition of maize straws. Soil Biol Biochem 38:2328-2335

Luna-Guido ML, Vega-Estrada J, Ponce-Mendoza A, Hernandez-Hernandez H, Montes-Horcasitas MC, Vaca-Mier M, Dendooven L (2003) Mineralization of ^{14}C-labelled maize in alkaline saline soils. Plant Soil 250:29-38

Mamilov A, Dilly OM, Mamilov S, Inubushi K (2004) Microbial eco-physiology of degrading aral sea wetlands: Consequences for C-cycling. Soil Sci Plant Nutr 50:839-842

Muhammad S, Müller T, Joergensen RG (2006) Decomposition of pea and maize straw in Pakistani soils along a gradient in salinity. Biol Fertil Soils 43:93-101

Muhammad S, Müller T, Joergensen RG (2008) Relationships between soil biological and other soil properties in saline and alkaline arable soils from the Pakistani Punjab. J Arid Environ (doi: 10.1016/j.jaridenv.2007.06.016)

Nelson PN, Ladd JN, Oades JM (1996) Decomposition of ^{14}C labelled plant material in salt-affected soil. Soil Biol Biochem 28:433-441

Nicolardot B, Fauvet G, Cheneby D (1994) Carbon and nitrogen cycling through soil microbial biomass at various temperatures. Soil Biol Biochem 26:253-261

Pankhurst CE, Yu S, Hawke BG, Harch BD (2001) Capacity of fatty acid profiles and substrate utilization patterns to describe differences in soil microbial communities associated with increased salinity or alkalinity at three locations in South Australia. Biol Fertil Soils 33:204-217

Pathak H, Rao DLN (1998) Carbon and nitrogen mineralisation from added organic matter in saline and alkali soils. Soil Biol Biochem 30:695-702

Potthoff M, Joergensen RG, Wolters V (2001) Short-term effects of earthworm activity and straw amendment on the microbial C and N turnover in a remoistened arable soil after summer drought. Soil Biol Biochem 33:583-591

Qadir M, Ghafoor A, Murtaza G (2000) Amelioration strategies for saline soils: a review. Land Degrad Develop11:501-521

Raubuch M, Roose K, Warnstorff K, Wichern F, Joergensen RG (2007) Respiration pattern and microbial use of field-grown transgenic *Bt*-maize residues. Soil Biol Biochem 39:2380-2389

Rasul G, Appuhn A, Müller T, Joergensen RG (2006) Salinity-induced changes in the microbial use of sugarcane filter cake added to soil. Appl Soil Ecol 31:1-10

Rasul G, Khan AA, Khan KS, Joergensen RG (2008a) Immobilization and mineralization of nitrogen during microbial use of sugarcane filter cake amended with glucose in a saline alkaline soil. Biol Fertil Soils (submitted)

Rasul G, Müller T, Joergensen RG (2008b) Soil microbial response to sugarcane filter cake and biogenic waste compost. Journal of Plant Nutrition and Soil Science (in press)

Rietz DN, Haynes RJ (2003) Effects of irrigation-induced salinity and sodicity on soil microbial activity. Soil Biol Biochem 35:845-854

Robertz M, Muckenheim T, Eckl S, Webb L (1999) Kostengünstige Labormethode zur Bestimmung der mikrobiellen Bodenatmung nach DIN 19737. Wasser Boden 51/5:48-53

Salamanca EF, Raubuch M, Joergensen RG (2006) Microbial reaction of secondary tropical forest soils to the addition of leaf litter. Appl Soil Ecol 31:53-61

Sardinha M, Müller T, Schmeisky H, Joergensen, RG (2003) Microbial performance in soils along a salinity gradient under acidic conditions. Appl Soil Ecol 23:237-244

Sindhu MA, Cornfield AH (1967) Comparative effects of varying levels of chlorides and sulphates of sodium, potassium, calcium, and magnesium on ammonification and nitrification during incubation of soil. Plant Soil 27:468-472

Sparrow SD, Lewis CE, Knight CW (2006) Soil quality response to tillage and crop residue removal under subarctic conditions. Soil Till Res 91:15-21

Swift MJ, Heal OW, Anderson, JM (1979) Decomposition in terrestrial ecosystems. Blackwell, Oxford

Theenhaus A, Maraun M, Scheu S (1997) Substrate-induced respiration in forest and arable soils measured by O_2-microcompensation: moisture conditions and respiratory quotient. Pedobiol 41:449-455

Tripathi S, Kumari S, Chakraborty A, Gupta A, Chakrabarti K, Bandyapadhyay BK (2006) Microbial biomass and its activities in salt-affected coastal soils. Biol Fertil Soils 42:273–277

Vance ED, Brookes PC, Jenkinson DS (1987) An extraction method for measuring soil microbial biomass C. Soil Biol Biochem 19:703-707

Wichern J, Wichern F, Joergensen RG (2006) Impact of increased salinity on soil microbial communities and decomposition of maize in acidic soils. Geoderma 137:100-108

Witter E, Kanal A (1998) Characteristics of the soil microbial biomass in soils from a long-term field experiment with different levels of C input. Appl Soil Ecol 10:37-49

Wu J, Joergensen RG, Pommerening B, Chaussod R, Brookes PC (1990) Measurement of microbial biomass C by fumigation extraction – an automated procedure. Soil Biol Biochem 22:1167-1169

Yuan BC, Li ZZ, Liu H, Gao M, Zhang YY (2007) Microbial biomass and activity in salt affected soils under arid conditions. Appl Soil Ecol 35:319-328

Zahran HH (1997) Diversity, adaptation and activity of the bacterial flora in saline environments. Biol Fertil Soils 25:211-223

Zhao XR, Lin Q, Brookes PC (2005) Does soil ergosterol concentration provide a reliable estimate of soil fungal biomass? Soil Biol Biochem 37:311-317

Chapter 6 – Summary

Five laboratory incubation experiments were carried out to assess the salinity-induced changes in the microbial use of sugarcane filter cake added to soil. The first laboratory experiment was carried out to prove the hypothesis that the lower content of fungal biomass in a saline soil reduces the decomposition of a complex organic substrate in comparison to a non-saline soil under acidic conditions. Three different rates (0.5, 1.0, and 2.0%) of sugarcane filter cake were added to both soils and incubated for 63 days at 30°C. In the saline control soil without amendment, cumulative CO_2 production was 70% greater than in the corresponding non-saline control soil, but the formation of inorganic N did not differ between these two soils. However, nitrification was inhibited in the saline soil. The increase in cumulative CO_2 production by adding filter cake was similar in both soils, corresponding to 29% of the filter cake C at all three addition rates. Also the increases in microbial biomass C and biomass N were linearly related to the amount of filter cake added, but this increase was slightly higher for both properties in the saline soil. In contrast to microbial biomass, the absolute increase in ergosterol content in the saline soil was on average only half that in the non-saline soil and it showed also strong temporal changes during the incubation: A strong initial increase after adding the filter cake was followed by a rapid decline. The addition of filter cake led to immobilisation of inorganic N in both soils. This immobilisation was not expected, because the total C-to-total N ratio of the filter cake was below 13 and the organic C-to-organic N ratio in the 0.5 M K_2SO_4 extract of this material was even lower at 9.2. The immobilisation was considerably higher in the saline soil than in the non-saline soil. The N immobilisation capacity of sugarcane filter cake should be considered when this material is applied to arable sites at high rations.

The second incubation experiment was carried out to examine the N immobilizing effect of sugarcane filter cake (C/N ratio of 12.4) and to investigate whether mixing it with compost (C/N ratio of 10.5) has any synergistic effects on C and N mineralization after incorporation into the soil. Approximately 19% of the compost C added and 37% of the filter cake C were evolved as CO_2, assuming that the amendments had no effects on the decomposition of soil organic C. However, only 28% of the added filter cake was lost according to the total C and $\delta^{13}C$ values. Filter cake and compost contained initially significant concentrations of inorganic N, which was nearly completely immobilized between day 7 and 14 of the incubation in most cases. After day 14, N re-mineralization

occurred at an average rate of 0.73 µg N g^{-1} soil d^{-1} in most amendment treatments, paralleling the N mineralization rate of the non-amended control without significant difference. No significant net N mineralization from the amendment N occurred in any of the amendment treatments in comparison to the control. The addition of compost and filter cake resulted in a linear increase in microbial biomass C with increasing amounts of C added. This increase was not affected by differences in substrate quality, especially the three times larger content of K_2SO_4 extractable organic C in the sugarcane filter cake. In most amendment treatments, microbial biomass C and biomass N increased until the end of the incubation. No synergistic effects could be observed in the mixture treatments of compost and sugarcane filter cake.

The third 42-day incubation experiment was conducted to answer the questions whether the decomposition of sugarcane filter cake also result in immobilization of nitrogen in a saline alkaline soil and whether the mixing of sugarcane filter cake with glucose (adjusted to a C/N ratio of 12.5 with $(NH_4)_2SO_4$) change its decomposition. The relative percentage CO_2 evolved increased from 35% of the added C in the pure 0.5% filter cake treatment to 41% in the 0.5% filter cake +0.25% glucose treatment to 48% in the 0.5% filter cake +0.5% glucose treatment. The three different amendment treatments led to immediate increases in microbial biomass C and biomass N within 6 h that persisted only in the pure filter cake treatment until the end of the incubation. The fungal cell-membrane component ergosterol showed initially an over-proportionate increase in relation to microbial biomass C that fully disappeared at 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 comparison to the control treatment. Since day 14, the immobilized N was re-mineralized at rates between 1.31 and 1.51 µg N g^{-1} soil d^{-1} in the amendment treatments and was thus more than doubled in comparison with the control treatment. This means that the re-mineralization rate is independent from the actual size of the microbial residues pool and also independent from the size of the soil microbial biomass. Other unknown soil properties seem to form a soil-specific gate for the release of inorganic N.

The fourth incubation experiment was carried out with the objective of assessing the effects of salt additions containing different anions (Cl^-, SO_4^{2-}, HCO_3^-) on the

microbial use of sugarcane filter cake and dhancha leaves amended to inoculated sterile quartz sand. In the subsequent fifth experiment, the objective was to assess the effects of inoculum and temperature on the decomposition of sugar cane filter cake. In the fourth experiment, sugarcane filter cake led to significantly lower respiration rates, lower contents of extractable C and N, and lower contents of microbial biomass C and N than dhancha leaves, but to a higher respiratory quotient RQ and to a higher content of the fungal biomarker ergosterol. The RQ was significantly increased after salt addition, when comparing the average of all salinity treatments with the control. Differences in anion composition had no clear effects on the RQ values. In experiment 2, the rise in temperature from 20 to 40°C increased the CO_2 production rate by a factor of 1.6, the O_2 consumption rate by a factor of 1.9 and the ergosterol content by 60%. In contrast, the contents of microbial biomass N decreased by 60% and the RQ by 13%. The effects of the inoculation with a saline soil were in most cases negative and did not indicate a better adaptation of these organisms to salinity. The general effects of anion composition on microbial biomass and activity indices were small and inconsistent. Only the fraction of 0.5 M K_2SO_4 extractable C and N in non-fumigated soil was consistently increased in the 1.2 M $NaHCO_3$ treatment of both experiments. In contrast to the small salinity effects, the quality of the substrate has overwhelming effects on microbial biomass and activity indices, especially on the fungal part of the microbial community.

Chapter 7 – Zusammenfassung

Fünf Inkubationsexperimente wurden im Labor durchgeführt, um die versalzungs-induzierten Veränderungen in der mikrobiellen Nutzung von Zuckerrohr-Filterkuchen im Boden abzuschätzen. Das erste Laborexperiment wurde durchgeführte, um die Hypothese zu prüfen, dass der niedrigere Gehalt an Pilzbiomasse in einem salzhaltigen Boden die Zersetzung einer komplexen organischen Substanz reduziert im Vergleich zu einem nicht-salzhaltigen Boden unter sauren Bedingungen. Drei verschiedene Anteile (0.5, 1.0 and 2.0%) des Zuckerrohr-Filterkuchens wurden zu beiden Böden gegeben und für 63 Tage bei 30°C inkubiert. In dem salzhaltigen Kontrollboden ohne Zugabe war die kumulative CO_2-Produktion um 70% höher als in dem entsprechenden nicht-salzhaltigen Kontrollboden, aber die Bildung von anorganischem N unterschied sich zwischen den beiden Böden nicht. Jedoch war die Nitrifikation in dem salzhaltigen Boden gehemmt. Die Zunahme der kumulativen CO_2-Produktion durch die Zugabe des Filterkuchens war in beiden Böden ähnlich und entsprach 29% des Filterkuchen-Kohlenstoffs bei allen drei Zugabemengen. Auch die Zunahmen an C und N in mikrobieller Biomasse waren linear mit der Menge des zugegebenen Filterkuchens korreliert, aber diese Zunahme war für beide Eigenschaften etwas höher im salzhaltigen Boden. Im Gegensatz zur mikrobiellen Biomasse war die absolute Zunahme des Ergosterolgehalts im salzhaltigen Boden nur halb so hoch wie im nicht-salzhaltigen Boden und zeigte große zeitliche Veränderungen während der Inkubation. Einer starken anfänglichen Zunahme nach der Zugabe des Filterkuchens folgte eine rasche Abnahme. Die Zugabe von Filterkuchen führte zu einer Immobilisation von anorganischem N in beiden Böden. Diese Immobilisation war nicht erwartet worden, da das C_t/N_t-Verhältnis des Filterkuchens unter 13 lag und das C_{org}/N_{org}-Verhältnis im 0.5 M K_2SO_4-Extrakt dieses Materials war mit 9.2 sogar noch niedriger. Die N-Immobilisationskapazität von Zuckerrohr-Filterkuchen sollte berücksichtigt werden, wenn dieses Material in großen Mengen auf ackerbaulich genutzten Flächen ausgebracht wird.

Der zweite Inkubationsversuch wurde durchgeführt, um den N-Immobilisationseffekt von Zuckerrohr-Filterkuchen (C/N-Verhältnis von 12.4) zu prüfen und um zu untersuchen, ob das Beimischen mit Kompost (C/N-Verhältnis von 10.5) synergistische Effekte auf die C- und N-Mineralisation nach der Inkorporation in den Boden hat. Ungefähr 19% des zugeführten Kompost-Kohlenstoffs und 37% des Filterkuchen-Kohlenstoffs wurden als CO_2 abgegeben. Dabei wurde angenommen, dass die Zugabe

keinen Einfluss auf den Abbau des organischen Kohlenstoffs im Boden hat. Allerdings wurden nur 28% des zugegebenen Filterkuchens auf der Basis der C_t- und $\delta^{13}C$-Werte abgebaut. Filterkuchen und Kompost enthielten anfänglich signifikante Konzentrationen an anorganischem N, die zwischen dem 7. und 14. Tag der Inkubation in den meisten Fällen nahezu vollständig immobilisiert wurden. Nach 14 Tagen setzte in den meisten Zugabevarianten die N-Remineralisation mit einer durchschnittlichen Rate von 0.73 µg N g^{-1} Boden d^{-1} ein. Damit entsprach diese ohne signifikanten Unterschied der N-Mineralisationsrate in der zugabelosen Kontrolle. Eine signifikante Netto-N-Mineralisation von organischem N erfolgte in keiner der Zugabevarianten im Vergleich mit der Kontrolle. Die Zugabe von Kompost und Filterkuchen resultierte in einem linearen Anstieg von C in mikrobieller Biomasse mit steigender Menge an zugeführtem C. Diese Zunahme wurde nicht durch die unterschiedliche Substratqualität beeinflusst, z.B. dem dreifach höheren Gehalt an K_2SO_4-extrahierbarem organischen C des Zuckerrohr-Filterkuchens. In den meisten Zugabevarianten nahmen C und N in der mikrobiellen Biomasse bis ans Ende der Inkubation zu. Keine Synergieeffekte konnten in den Mischungsvarianten von Kompost und Zuckerrohr-Filterkuchen beobachtet werden.

Der dritte, 42-tägige Inkubationsversuch wurde durchgeführt, um die Fragen zu beantworten, ob die Zersetzung von Zuckerrohr-Filterkuchen ebenfalls in einem salzhaltigen und alkalischen Boden zu einer Immobilisation von N führt und ob das Mischen von Zuckerrohr-Filterkuchen mit Glucose (eingestellt auf ein C/N-Verhältnis von 12.5 mit $(NH_4)_2SO_4$) die Zersetzung beeinflusst. Der relative Anteil der CO_2-Produktion nahm von 35% der zugegebenen C-Menge in der reinen 0.5% Filterkuchen-Variante über 41% in der 0.5% Filterkuchen +0.25% Glucose-Variante auf 48% in der 0.5% Filterkuchen +0.5% Glucose-Variante zu. In den drei Zugabevarianten kam es zu einer unmittelbaren Zunahme von C und N in mikrobieller Biomasse innerhalb von 6 h, die nur in der reinen Filterkuchen-Variante bis ans Ende der Inkubation anhielt. Die pilzliche Zellmembran-Komponente Ergosterol zeigte anfänglich einen überproportionalen Anstieg in Relation zum C in mikrobieller Biomasse, der vollständig bis zum Ende der Inkubation verschwand. Die Cellulase-Aktivität zeigte eine fünffache Zunahme nach Filterkuchen-Zugabe, die nicht durch eine weitere Glucosezugabe erhöht wurde. Die Cellulase-Aktivität zeigte eine exponentielle Abnahme auf ein Niveau um 4% des Ausgangswerts in allen Varianten. Die Menge an anorganischem N, die von Tag 0 bis Tag 14 immobilisiert wurde, nahm mit zunehmender Menge an C im Vergleich mit der Kontrollvariante zu. Ab dem Tag 14 wurde das immobilisierte N mit Raten zwi-

schen 1.31 und 1.51 µg N g^{-1} Boden d^{-1} in den Zugabevarianten remineralisiert und war damit mehr als doppelt so hoch wie in der Kontrollvariante. Das bedeutet, dass die Remineralisationsrate unabhängig von der aktuellen Größe des Pools an mikrobiellen Residuen ist und damit ebenso unabhängig von der Größe der mikrobiellen Biomasse. Andere unbekannte Bodeneigenschaften scheinen einen boden-spezifischen Regulator für die Freigabe von anorganischem N zu bilden.

Der vierte Inkubationsversuch wurde mit Absicht durchgeführt, die Auswirkungen von Salzzugaben mit unterschiedlicher Anionenzusammensetzung (Cl^-, SO_4^{2-}, HCO_3^-) auf die mikrobielle Nutzung von Zuckerrohr-Filterkuchen und Dhancha-Blättern, die zu inokuliertem sterilen Quarzsand zugegeben wurden, zu untersuchen. In dem fünften Versuch war die Absicht, die Auswirkungen von Inokulum und Temperatur auf die Zersetzung von Zuckerrohr-Filterkuchen abzuschätzen. In dem vierten Experiment führte der Zuckerrohr-Filterkuchen zu signifikant niedrigeren Respirationsraten, niedrigeren Gehalten an extrahierbarem C und N, und niedrigeren Gehalten an C und N in mikrobieller Biomasse als die Dhancha-Blätter, aber zu höheren respiratorischen Quotienten RQ und zu höheren Gehalten des pilzlichen Biomarkers Ergosterol. Der RQ war signifikant größer nach Salzzugabe, wenn der Mittelwert aller Salzzugabe-Varianten mit der Kontrolle verglichen wurde. Unterschiede in der Anionenzusammensetzung hatten keine klaren Auswirkungen auf die RQ-Werte. Im fünften Experiment, erhöhte die Zunahme der Temperatur von 20 auf 40°C die CO_2-Produktionsrate mit einem Faktor von 1.6, O_2-Verbrauchs mit einem Faktor von 1.9 und den Ergosterolgehalt um 60%. Im Gegensatz dazu nahm der Gehalt an N in mikrobieller Biomasse um 60% und der RQ um 13% ab. Das Inokulum mit salzhaltigem Boden hatten in den meisten Fällen negative Auswirkungen und zeigte keine bessere Anpassung dieser Organismen an Salinität. Die generellen Auswirkungen der Anionenzusammensetzung auf die Indizes für mikrobielle Biomasse und Aktivität waren gering und inkonsistent. Nur die Fraktion des 0,5 M K_2SO_4-extrahierbaren Kohlenstoffs und Stickstoffs in nicht-fumigierten Böden war beständig erhöht in der 1,2 M $NaHCO_3$-Variante von beiden Experimenten. Im Gegensatz zu den geringen Salinitäts-Effekten hatte die Substratqualität überwältigenden Auswirkungen auf die Indizes für mikrobielle Biomasse und Aktivität, insbesondere auf den pilzlichen Anteil der mikrobiellen Gemeinschaft.

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