Department of Soil Biology and Plant Nutrition
Faculty of Organic Agricultural Sciences

Microbial use of organic substrates and maize growth, especially in saline and alkaline soils of the Pakistani Punjab

Dissertation

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by

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Witzenhausen, February 2005

Dedicated

to

My parents

and

My loving wife Tasneem and sons Muhammad and Fahad

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General Introduction

1. Background

World agricultural growth has slowed down after the past three decades. It was 3% a^{-1} in the 1960s, 2.3% a^{-1} in the 1970s and 2% a^{-1} in the period from 1980 to 1992. This trend has given rise to concern about the capability of world agriculture production to keep up with the growth of world population. It has raised the issue of world food security, particularly for the vulnerable regions in the developing countries. The growth rate of gross world agricultural output may slow down further to 1.8% a^{-1} by 2010. On the other hand, there has been unprecedented growth in world population over the past decades. The world population at the start of the 20th century was approximately 1.6 billion and reached a level of 2.52 billion in 1950. It is about 6 billion at this stage and there are projections that it will reach 8 billion before the year 2020 and as much as 9.322 billion in 2050. Similarly, China is projected to reach 1.462 billion, India 1.572 billion, USA 397.1 million, Pakistan 344.2 million and Bangladesh 265.4 million. This will put a lot of pressure on land to produce more food and fibre per unit of land to match requirements of growing population all over the world (Alam and Naqvi, 2003).

Presently, Pakistan is in the grip of a massive population explosion and has experienced a greater population growth compared to other developing countries. The population of Pakistan is increasing at 2.61% p.a. and the gap between the supply and demand of agricultural products is widening day by day. The population was about 33.82 million in 1951 and just 20 years later it had increased to 65 million. The current population of Pakistan is above 145 million, but it may touch 180 million by 2010 and even 344.2 million in 2050 (Alam and Naqvi, 2003). Increasing population pressure on quantity and quality in food supply and dwindling land and water resources force

agriculture to steadily increase the productivity of land through higher yields and crop intensity. To meet the challenge to food supply posed by the burgeoning population of Pakistan, there is an urgent need to boost crop yield. The issues in developing countries are growing population, fragile food security, and low agriculture inputs like fertilizer, poor yields, degrading soils and dependence on imports from the developed world. On the other hand, the land availability per capita will decline. 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.

The total geographical area of Pakistan is 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. Most of the areas in the Punjab and Sindh provinces comprised plain land, formed by the River Indus. Pakistan is known for its excellent network of irrigation canals and rich agricultural lands, with three major reservoirs - Tarbela, Mangla and Chashma, 23 barrages, 12 huge inter-river irrigation canals, 59,200 km of irrigation canals and more than 106 km of water courses and 107,000 field channels. About 1.6 million km are covered by water courses, farm channels and field ditches. In addition, the irrigation infrastructure is supplemented by 43.4 million acre feet (MAF) of groundwater pumping. The huge network of irrigation serves 43 command areas with 45,000 villages, covering 16.22 million ha of the national cultivated and cropped area of 22 million ha. A remarkable 82% of the agricultural area in Pakistan is covered by irrigation.

Pakistan consists of four provinces, Punjab, Sindh, NWFP and Balochistan plus federally administered Tribal Areas. The economy of the country is basically agrarian and is heavily dependent on irrigation, largely confined to the Indus Plain. The country is part of the sub-continent south of the Himalayan mountains situated between

longitude 61° and 76° E and latitude 24° and 37° N. The climate in Pakistan is arid to semi-arid, with temperatures ranging between 2°C and 50°C. The mean annual precipitation ranges from less than 10 cm to more than 75 cm. There are great variations in the soils of Pakistan.

2. Importance of agriculture for Pakistan

Pakistan is basically an agricultural country and its economy is mainly agrarian. Agriculture is the biggest sector of the economy and accounts for about 35 to 40% of the national income. Pakistan, like many developing countries of the world, is faced with the problem of low agricultural productivity. Many countries including Pakistan are faced with the challenge of producing more food and fibre, while there is little room for expansion in the cultivated area, and yield per unit area of various crops is very low, in spite of the fact that our country is blessed with a wide range of climate, soil conditions and irrigation water. The country is totally dependent on agriculture for the supply of food and fibre. Therefore, it is imperative to increase food and fibre production not only to cope with ever growing requirements of the country, but also for the sake of foreign exchange earnings and achieving self-sufficiency. Rapid agricultural growth can stimulate and thus sustain the pace of industrial growth, thus setting into motion a mutually reinforcing process of sustained economic growth.

In Pakistan, agriculture is the largest income generating sector, and employing more than 45% of the country's total labour 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. Apart from the sector’s immediate economic contribution, it also has indirect links with various

parts of the economy. Any changes in agricultural productivity, therefore, send a ripple effect throughout the rural population of Pakistan, 67.5% of whom derive their sustenance from agriculture in some way or other. 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.

The agriculture framework in Pakistan is supported to a great extent by the crop sector, with its percentage contribution to agriculture GDP exceeding that of other sectors. In 2000/01, the crop sector contributed 40% to agricultural GDP, as opposed to a 38% contribution from livestock and 5% from the fisheries and forestry sectors. The agriculture sector grew by 2.6% in 2003/04 which is lower than actual growth of 4.1% last year and a target of 4.2%. Major crops, accounting for 34% of agricultural value added, grew by 2.8%, against a 6.9% rise in value addition for last year and a target of 5.5% for 2003/04. Minor crops, which contribute 12% of value addition in agriculture, grew by 1.7% in 2003/04, against a growth target of 3.5% and a slight increase of 0.4% last year. The Livestock sub-sector, which accounts for almost one half of overall value addition in the agricultural sector (49%), has witnessed a modest growth of 2.6% in 2003/04, against a target of 3.0% and an actual achievement of 2.8% last year.

Pakistan's economy has undergone considerable diversification over the years, and yet the agricultural sector was still the largest sector in 2003/04. With its present contribution to GDP at 23.3%, it accounts for 42.1% of the total employed labour force and is the largest source of foreign exchange earnings by serving as the base sector for the country's major industries like textile and sugar. It also contributes to growth by providing raw materials as well as being a market for industrial products. What happens to agriculture is thus bound to have a substantial impact on the growth of overall GDP. Over the last decade, i.e. the 1990s, agriculture grew at an annual average rate of 4.5%

per annum, but was quite volatile, rising as high as 11.7% and declining by as much as 5.3%. The overall performance of agriculture during (2000/01 and 2001/02) was depressed as it was adversely affected by the unprecedented drought situation. Agricultural growth showed a negative trend for these two years. However, during 2002/03, the extent of water shortage was relatively less and Agriculture grew by 4.1%. During 2003/04, the widespread rains and increased snowfall in the catchment areas contributed to an improvement in the water situation as agriculture grew by 2.6%. Slower growth in agriculture during 2003/04 is mainly attributed to a decline in production of cotton followed by the slower growth in livestock, the largest contributor to agricultural growth. Furthermore, the shifting of slaughtering from livestock to manufacturing also slowed livestock growth and hence agricultural growth. Likewise, a 2% growth of fisheries against the 3.4% growth last year was due to the oil spill of the Tasman Spirit at the Karachi Port which killed millions of fish and contributed to the slower growth of agriculture.

As stated earlier, the shortage of water is receding. The canal head withdrawal in the Kharif (summer cropping season), 2003 and Rabbi (winter cropping season), 2003/04 seasons increased by 4.96% and 26.16%, respectively over the Kharif 2002 and Rabi 2002/03. Moreover, the heavy snowfall on the mountains during winter 2003 will help fill the country's water reservoirs and alleviate water shortages to a greater extent for the Kharif Crops (summer cropping season) 2004. On the whole, the water situation in the current fiscal year appears better than last year but is still lower than normal supplies.

The agricultural growth is estimated at 2.6% a^{-1} during 2003/04. Major crops, accounting for 34.2% of agriculture value added, grew by 2.8% a^{-1}, against 6.9% last year. Minor crops, contributing 12.4% to agricultural value added, registered a weaker growth of 1.7%, against 0.4% last year. Livestock, the largest contributor to overall

agriculture value added (contributing 49.1%), grew by 2.6% in 2003/04, as against 2.8% in 2002/03. Fisheries, accounting for 1.4% of agriculture value added, have shown a growth of 2%, against a growth of 3.4% last year. On the other hand, forestry, contributing 2.9% to agricultural value added, grew by 2.9% as against growth of 11.1% last year.

Amongst the major crops, wheat production is estimated at 19.767 million tons in 2003/04, as against 19.183 million tons last year, showing an increase of 3%. Rice production is estimated at 4.848 million tons in 2003/04, against 4.478 million tons last year, an increase of 8.3%. Sugarcane (*Saccarum officinarum* L.) production has increased by 2.6% in 2003/04, from 52.056 million tons last year to 53.419 million tons in 2003/04. Cotton production has, however, decreased from 10.211 million bales in 2002/03 to 10.048 million bales in 2003/04, showing a decrease of 1.6%. As regards the minor crops, the production of two major pulses, namely lentil *(Lens culinaris)* and mung *(Vigna mungo)* have increased this year. Production of lentil has increased by 2.7%, followed by mung *(Vigna mungo)* (1.7%) during 2003/04. The production of mash declined by 13.7%. The production of onion is estimated to increase by 16.1%. The production of chillies (*Capsicum annum*) and potato decreased by 29.5% and 4.7%, respectively in 2003/04 over the last year. Agriculture credit disbursement of Rs. 47.925 billion during July to March 2003/04, is higher by 27.3%, as compared to Rs. 37.632 billion over the corresponding period last year. The fertilizer off-take stood at 2508 thousand nutrient tons in July to March 2003/04 or higher by 9.5%, as compared to 2291 thousand nutrient tons for the corresponding period last year.

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. Pakistani agriculture, with the advent of Green Revolution technologies, has been one of the striking success stories of the post-independence era.

The introduction of the Green Revolution, beginning with the rice and wheat revolutions in the late 1960s, and extending to several other crops including oilseeds in recent years, ushered in an era of food self-sufficiency and improved rural welfare of the country.

Notwithstanding remarkable achievements on the food and agriculture front, several weaknesses persist and future challenges are complex and daunting. The yields of crops in Pakistan are comparatively lower than those 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, low yield per acre unit and traditional and old 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. In this study, the main thrust is to seek and develop new strategies and methodologies for the amelioration of saline and alkaline areas of Pakistan, which is an increasingly serious problem. Every year, a lot of cultivated fertile land is going out of cultivation due to the menace of salinity and alkalinity.

Pakistan is a land of promise and tremendous development possibilities by virtue of its unique geographical location, fast acquisitional talents of its people, and richness of natural and cultural resources. Most of the land area of Pakistan is classified as arid to semi-arid because rainfall is not sufficient to grow agricultural crops, forest and fruit plants and pastures. The cultivable area of Pakistan is 35.4 million ha, forest land accounts for 3.5 million ha, cultivable waste 8.6 million ha, cultivated area 22 million ha, the waterlogged and salt affected area in the Indus Basin is 6.8 million ha, and the

salt affected area outside the Indus Basin is 6.3 million ha. The hot deserts extend over some western areas and Thar, Cholistan and Thal. The Thar and Cholistan are part of the Great Indian Desert and cover the area east of the southern half of the Indus plains. The Thal area is between the Jhelum and Indus Rivers. Agricultural production in Pakistan is still three to four times less than developed countries like USA, Japan, Holland, France, UK, etc. The total cultivated area increased from 19.2 million ha in 1965 to 22.0 million ha in 2004 (Economic Survey of Pakistan, 2003/04). Since the area under cultivation cannot be increased significantly, due attention has to be paid to mechanical as well as other inputs in order to meet the problems of food, fibre and shelter for the growing population of Pakistan.

3. Salinity hazard effect on crop productivity

There are many reasons for the decreasing productivity of the agricultural crop. Soil fertility is decreasing day by day due to intensive cropping in order to fulfil the needs of the rapidly growing population. To maintain the fertility status of soils in order to supply adequate nutrients for plants, application of different fertilizers is recommended by agricultural scientists. Like many other parts of the world, salinity and water logging are the major constraints on crop production in Pakistan. Of the 22 million ha of total cultivable land, 6.3 million ha are salt-affected. Soil salinity may be robbing Pakistan of about 30% of its potential production of major crops. A major part of salt-affected soils (about 3.5 million ha) are presently cultivated to rice, wheat, cotton, sugarcane, rape seed and other crops with a substantial reduction in yield. According to an estimate, there is a net yearly addition of 0.98 to 2.47 t ha^{-1} through various sources and each year 0.20 to 0.40% of the total arable land is going out of cultivation because of salinity.

Saline soils cover about 380 to 995 million ha of the Earth's land surface (Tanji, 1990; Szabolcs, 1994; IAEA, 1995) and, of these, 62% are saline-sodic or sodic. This area is expected to increase in the future and more effort and economic resources will be needed to cope with the problem, which is especially important in developing countries where yield stability is critical for subsistence of population (Flowers and Yeo, 1995). The estimates of salt-affected area in Pakistan vary widely because of the different classification criteria and survey methods used by various agencies (Sandhu and Qureshi, 1986; Ghassemi et al., 1995). However, about 6.3 million ha are believed to be salt affected, and the ground water in most of these saline area is brackish and thus unfit for irrigation (Qurashi and Baarret-Lennard, 1998). A soil salinity survey (Water and power Development Authority, 1985) has indicated that 38% of soil profiles studied were salt affected; of these 24% were saline sodic, 11% saline and 3% non-saline-sodic.

Accumulation of excess sodium salt (Na^+) in soil causes numerous adverse phenomena, such as changes in the exchangeable and soil solution ions and soil pH, destabilization of soil structure, deterioration of soil hydraulic properties, increased susceptibility to crusting and specific ion effects on plants (Shainberg and Levy, 1992; Qadir and Schubert, 2002). Saline-sodic soils slake, disperse and swell under specific conditions when wet with rain or irrigation water; this decreases water and air movement, plant-available water, root penetration, seedling growth and plant establishment and increases surface runoff, pounding, water-logging, erosion and impedes seed bed preparation (Sumner, 1993; Rengasamy and Sumner, 1998; Oster et al., 1999). Soil microbial aspects of saline, alkaline and sodic environments have been meagrely studied (Zahran, 1997), but recent studies clearly revealed the adverse effects of salinisation on the microbial biomass (Sarig et al., 1996; Batra and Manna, 1997; Rietz and Haynes, 2003). In particular, the fungal part of the microbial biomass estimated by PLFA (Badran, 1994; Pankhurst et al., 2001) or ergosterol analysis

(Sardinha et al; 2003) was strongly reduced under saline soil conditions. In contrast the effect of salinisation on C and N mineralization or added plant material is contradictory, i.e. both increases (Nelson et al., 1996) and decreases (Pathak and Rao, 1998) have been reported. It is still not clear whether microbial processes that are crucial for decomposition of crop residues and other organic amendments in soil and thus for the release of nutrients to sustain productivity are affected by salinisation and, if so, to what extent. In Pakistan, under arid and semiarid climate, salinity is usually combined with high pH conditions due to the presence of calcium carbonate in the uppermost soil layers (saline soils) or to hydrolysis of sodium carbonate (sodic soils).

4. Assessment of soil fertility in Pakistani soils

Soil microbial, chemical and physical characterization is indispensable for knowing the soil nutrient level and its nutrient supplying capability. The assessment of soil fertility, i.e. the ability of a soil to provide nutrients for plant growth to gain a certain crop yield, and quality, i.e. the capacity of a soil to maintain key ecological functions such as decomposition and formation of organic matter, are important objectives in tropical regions (Srivastava and Lal, 1994; Wick et al., 1998). Since temperature controls many processes in soil, especially microbiologically mediated ones, the higher temperatures in tropical regions lead to faster turnover rates of microbial biomass and soil organic matter in comparison to temperate Northern Europe, shortening the time taken for ecosystems to respond to changes in management practices (Grisi et al., 1998). In contrast to the nearly constant air temperature, humidity and soil water content are characterized by extreme changes between dry and rainy seasons, considerably affecting soil biological processes (Insam et al., 1989; Wardle, 1998).

The effects of extreme seasonal variations in water content have been investigated in highly weathered soils from old land surfaces in Africa (Wick et al., 1998), in soils from Gangetic alluvium in India (Srivastava and Lal. 1994), and from aged volcanic ash soils in Costa Rica (Mazzarino et al., 1993). No information exists on the biological properties of saline and alkaline soils from tropical and sub-tropical regions of Pakistan. The shallow water table of these regions often accentuates the salinity, due to high evapo-transpiration. These soils are dominated by soluble salts of Ca, Na, Mg and Cl, which are more detrimental to soil structure.

These salts play a very important role in de-flocculating the clay particles, soils become impermeable and ultimately infiltration and percolation of water ceases from the upper surface and within the soils. This salt is deposited on the surface of the soils, which impairs plant growth. Moreover, organic matter contents of these soils is very low and indicates less biological activity in these soils.

5. Importance of organic substrates in maintaining soil biological fertility

It is of prime importance to incorporate organic substrates to maintain soil fertility, productivity and soil organic matter (SOM) and counteract nutrient depletion in tropical regions like Pakistan. These soils are very much depleted due to intensive cropping and raising more and more crops over the year to fulfil the food requirement of growing population. Incorporation of plant residues 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. Plant residues are known to affect soil physical properties (Hulugalle et al., 1986), availability of nutrients (Wade and Sanchez, 1983) and soil biological

activity (Tian et al., 1993). However, effects of plant residues on soil and crops differ and depend on their decomposition and nutrient release rates. Ground or finely chopped residue material, for example, is likely to be more susceptible to microbial attack than intact plant parts due to a better soil residue contact (Angers and Recous, 1997) and lack of intact lignified barrier tissues (Summerell and Burgess, 1989). In contrast to this, however, fine particles are also more likely to be protected against decomposition through physical protection by clay and other particles (Stickler and Frederick, 1959). The rate of CO_2 efflux, under conditions where moisture and temperature are not limiting, can provide an indication of organic matter quality and whether the soil environment is conducive to the decomposition process (Sparling, 1997). Plant residues with a high C-to-N ratio and high lignin and polyphenol contents decompose and release nutrients slowly (Fox et al., 1990; Palm and Sanchez, 1991; Tian et al., 1992). Such residues have a low direct nutrient effect and a high indirect mulching effect on crops. In turn, residues with a low C-to-N ratio, and lignin and polyphenol contents decompose rapidly, and have a high direct nutrient effect and a low indirect mulching effect. Therefore, the decompositions of plant residue are related to their C-to-N ratio and lignin and phenol contents. To better predict the effect of plant residues on the soil and crop, a plant residue quality was proposed by Tian et al. (1995). It is very well documented that by the addition of organic substrates there is an increase in the microbial activities and biomass.

6. Specification and history of sampling sites

The Punjab has a total geographical area of 205,344 km^2 and is the second largest province of Pakistan on an area basis, but the largest on a population basis, with 80

million. The name Punjab is coined from two words; Punj means five and ab means water, in other words land with five rivers. The land of Punjab is mostly irrigated and 80% is under artificial irrigation by canal. The soils of Punjab vary from very fertile to non-fertile. Pakistani Punjab is divided into eight different regions, which are called Divisions. One division may consist of three to four districts and many villages. Agricultural land of every region has its own characteristics and cropping pattern regarding their physical and chemical properties. Special care is not taken during land use due to the ignorance of the illiterate farmer community. The selected divisions for sampling (Fig. 1) are described below with their typical characteristics and cropping pattern and specifications.

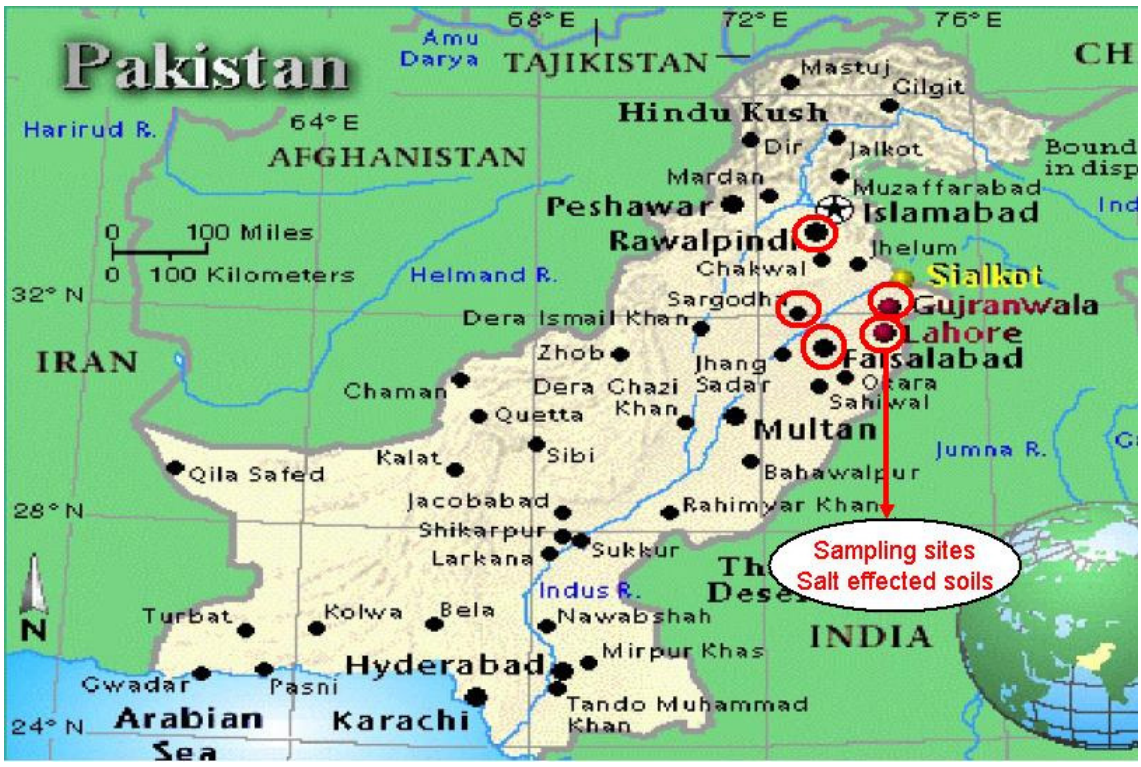

Fig. 1: Map of Pakistan and location of the selected divisions for sampling

6.1. Sargodha region

The total cultivated area of the Sargodha region is 26,358 km^2 (Federal Bureau of Statistics, 2003). It is a densely populated region of the Punjab and intensive cropping is done in this region. The frequent adopted cropping pattern for this region is sugarcane, wheat, tobacco, maize, and sorghum. Crop rotations including legumes are rare and exhaustive crops are often planted successively. Sugarcane is grown consistently for more than five years consecutively year after year, which causes severe nutrient depletion in the agricultural land. Mostly mineral fertilizers are added to these soils, like DAP (diammonium phosphate), urea and SSP (single super phosphate). The yield of these soils is very low, ranging from 800 kg to 1200 kg ha^{-1}. Fertile soils have a higher yield potential but due to mismanagement of farmers it is also not possible to get higher yields from these soils. Very little care is taken regarding their fertility maintenance. Moreover, very low soil organic matter accentuates the severity in nutrient supply to crops, which leads to poor stands of the major crops. Intensive cropping has even damaged their capacity for nutrient supply, further reducing nutrient levels. Addition of farmyard manure, compost or organic wastes is also very rare. So it is time to find alternative methods for improving soil fertility so that this soil can feed the rapidly growing population. Otherwise, it will be very difficult in the long-term to compensate for such a tremendous growth in population, with a rate of 2.7% a^{-1}.

6.2. Gujranwala and Lahore regions

The total cultivated areas of Gujranwala and Lahore regions are 17,213 km^2 and 16,104 km^2 (Federal Bureau of Statistics, 2003). These two regions are very famous for rice

cultivation and contributing about half of Pakistan's rice export to the world. If the fertility of these soils could be managed using new methods and technologies, then the farmers would be able to double the yield from these two regions, which would boost the national economy and increase the living standards of the poor farming communities of these regions by enhancing the export of rice. These regions have a very famous rice belt, which is known as the "Kaalar tract". This tract has slight to moderate salinity and sodicity with a high pH, which can cause a reduction in the yield of rice by up to 40 to 60% (Aslam, 1987). There is room for a biological approach because a lot of effort has been put into increasing soil productivity by chemical and physical amendments and improvements. These methods and technologies have benefited soils partially but not completely. Soils of these regions have specific characteristics for rice. The rice from these regions has a special aroma, which is due to its microclimatic conditions. About 75% of the Gujranwala cultivated area is salt affected and this varies from slightly saline to sever salinity. The cropping pattern of these regions is rice, wheat, and rice or rice, wheat, maize, and fodder legumes. Moreover, $ZnSO_4$ and Cartap are added to these soils for rice only. Cartap is used against the stem borer and $ZnSO_4$ against sterility and for better florescence. The yield of these regions ranges from normal to very low (850 kg ha^{-1}).

6.3. Faisalabad region

The total cultivated area of this region is 17,917 km^2 (Federal Bureau of Statistics, 2003) and is also one of the biggest agricultural regions of the Punjab famous for wheat, maize and sorghum cultivation. The pH of this region ranges from 9 to 10.5 and it also has very high electrical conductivity. Rice is also cultivated in some parts of the

Faisalabad region. The soil fertility of these soils is very low. The yield of this region is also very low and ranges from 900 to 1300 kg ha^{-1}. There is also heavy application of mineral fertilizers like DAP, urea and $ZnSO_4$.

6.4. Gujrat and Rawalpindi regions

The total cultivated area of these regions is 3,192 km^2 and 22,254 km^2 (Federal Bureau of Statistics, 2003). These regions are relatively barren, due to their undulating nature, which is why they mainly depend on precipitation. But the annual precipitation of these regions of up to 1177 mm a^{-1} is unevenly distributed throughout the year and is not enough to raise agricultural crops. More than half of the rainfall occurs in July, which is called the monsoon season. The cropping pattern of these regions is barley, maize, wheat, and sorghum. There is also a very low supply of basic inputs like mineral fertilizers because of poverty. The average yield of these regions is in the range of 750 to 900 kg ha^{-1}.

7. Objectives

1. Plant growth stimulates microbial growth and activity in the rhizosphere by exudates and a large variety of other rhizodeposits (Joergensen, 2000; Mayer et al., 2003), changing the mineralization of native soil organic matter (Dormaar, 1990; Kuzyakov, 2002). Consequently, also the decomposition of freshly incorporated plant residues must be altered in the rhizosphere of actively growing plants. Therefore, the first study was carried out to prove the following hypothesis: The

decomposition of alfalfa residues is enhanced by the presence of living plant roots, due to higher microbial activity and biomass in the rhizosphere.

2. In the second study, the central objective of the research was to analyse the interactions between the most important soil physical (texture), soil chemical (pH, salinity indices, soil organic matter, P status) and soil biological properties in Pakistani soils, investigating 30 typical alkaline and saline arable sites differing strongly in salinisation and in soil pH. The soil biological properties were differentiated into indices for microbial activity (basal respiration), microbial biomass (C, N, and P), and community structure (fungal ergosterol) with the aim of assessing their potential as soil fertility and soil quality indices in alkaline and saline arable soils. The hypotheses were: (1) Increasing salinity and increasing alkalinity have depressive effects on both microbial biomass and activity indices and (2) increasing salinity and increasing alkalinity both lead to strong changes in microbial community structure towards bacteria.

3. In the third experiment, three organic amendments (compost, maize straw and pea straw) differing in quality of the organic components and nutrient content, especially N and P were added to five alkaline Pakistani soils along a gradient in salinity to test the following two hypotheses: (1) Increasing salinity at high pH decreases proportionally the decomposition of the added organic amendments and consequently the net increase in microbial biomass. (2) Salinity effects override differences in substrate quality of the organic amendments.

4. It was the aim of the last experiment to investigate the interactions between plant growth, microbial biomass formation and compost decomposition. Therefore, an experiment was designed with different combinations of amending compost and triple super-phosphate, comparing a strongly saline Pakistani arable soil with a non-saline German arable soil.

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Chapter 2

Impact of growing maize (*Zea mays*) on the decomposition of incorporated fresh alfalfa residues

Impact of growing maize (*Zea mays*) on the decomposition of incorporated fresh alfalfa residues

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Key words: $\delta^{13}C$, particulate organic matter, microbial biomass, ergosterol, CO_2 evolution rate

Abstract

In this study, the effects of growing maize plants on the microbial decomposition of easily degradable plant residues was investigated in a 90-day pot experiment using a sandy arable soil. Four treatments were carried out: (1) untreated control, (2) with freshly chopped alfalfa residues (*Medicago sativa* L.) incorporated into soil, (3) with growing maize plants (*Zea mays* L.), and (4) with growing maize plants and freshly chopped alfalfa residues incorporated into soil. The amount of alfalfa residues was equivalent to 1.5 mg C g^{-1} soil and 120 µg N g^{-1} soil. Only the combination of growing maize plants and alfalfa residues significantly increased the contents of microbial biomass C, microbial biomass N, and ergosterol in soil compared to the control. The dry weight of the maize shoot material was more than doubled in the treatment with alfalfa residues than without. Assuming that the addition of alfalfa residues did not affect the decomposition of native soil organic matter, only 27% of the alfalfa residues were found as CO_2 using a portable gas analyser with a dynamic chamber. This suggests that a considerable part of alfalfa-C remained undecomposed in the soil. However, only 6% of the alfalfa residues could be recovered as plant remains > 2 mm in treatment 2. The reasons for this discrepancy are discussed. In the particulate organic matter (POM) fractions 63–400 µm and 400–2000 µm there are no indications that any alfalfa residues were transferred into this fraction. Based on $\delta^{13}C$ values, it was calculated that plant remains > 2 mm in treatment 4 contained 14.7% alfalfa residues and 85.3% maize root remains. This means that 54 µg g^{-1} soil or 60% more alfalfa-C was recovered in this treatment than in treatment 2. The reasons for this apparent retardation in the

decomposition of alfalfa residues and the problems of the methodological approach are discussed.

Introduction

The decomposition of plant litter by soil microorganisms is a central soil function leading to the release of nutrients for plant growth (Swift et al., 1979). However, our knowledge of interactions between plant growth and microbial decomposition of plant litter is very much restricted, due to severe methodological problems: (1) the measurements of microbial biomass and activity in the presence of living plants and (2) the differentiation between the growing plant and decomposing litter. However, the fumigation-extraction method with pre-extraction (Mueller et al., 1992; Mayer et al., 2003), especially in combination with ergosterol, allows a reliable estimation of the microbial biomass in the presence of living roots (Joergensen, 2000). The recovery of non-decomposed plant material by the simple sieving procedure of Magid and Kjaergaard (2001) and Magid et al. (2004) might be able to replace the litter-bag technique, which has important drawbacks in determining the decomposition of plant residues by soil organisms: the change of the microclimate and especially a reduced contact between plant residues and soil colloids (Fruit et al., 1999; Knacker et al., 2003). Due to the progress in determining differences in the natural $\delta^{13}C$ signature of C3 and C4 plants (Balesdent and Mariotti 1996), it should be possible to differentiate between the roots of a C4 plant and decomposing C3 litter and vice versa.

The C4 plant maize is relatively enriched with ^{13}C and leaves a rather bare soil surface due to the large individual plants, enabling the measurement of CO_2 in pots by the dynamic chamber method (Wichern et al., 2004). Alfalfa is an important plant for

improving soil fertility all over the world, including arid tropical countries (Wichern et al., 2004). It is an increasingly important plant for use as green manure in stockless organic agriculture (Müller et al., 2003). Alfalfa litter is also a very common material used to test the effects of hazardous chemicals on soil microbial decomposition processes (Anderson, 1992; Chen et al., 2001).

Plant growth stimulates microbial growth and activity in the rhizosphere via exudates and a large variety of other rhizodeposits (Joergensen, 2000; Mayer et al., 2003), changing the mineralization of native soil organic matter (Dormaar, 1990; Kuzyakov, 2002). Consequently, also the decomposition of freshly incorporated plant residues must be altered in the rhizosphere of actively growing plants. Therefore, the present study was carried out to prove the following hypothesis: The decomposition of alfalfa residues is enhanced by the presence of living plant roots, due to higher microbial activity and biomass in the rhizosphere.

Materials and methods

Soil sampling and preparation

An arable sandy soil was collected at 0-10 cm depth from the site Allerberg in the south of Göttingen, Germany, and sieved (< 2 mm mesh). A greenhouse pot experiment with 7 replications was conducted with the following four treatments: (1) control without maize plants and without alfalfa residues, (2) with freshly chopped alfalfa residues (*Medicago sativa* L.) incorporated into soil, (3) with growing maize plants (*Zea mays* L.), but without alfalfa residues, and (4) with growing maize plants and freshly chopped

alfalfa residues incorporated into soil. The experiment was started on 21 May 2001 and was carried out for 90 days until 19 August 2001 in a greenhouse.

Each treatment consisted of 10 kg soil (on an oven-dry basis), filled in pots of 25 cm height and adjusted to 60% water holding capacity by watering from the top and also adjusted to 1.3 g cm^{-3} bulk density by pressing the soil with a wooden hammer. In the two alfalfa residue treatments, 200 g of freshly harvested alfalfa were mixed as 3 cm pieces into 10 kg soil before filling into the pots. The amount of alfalfa residues (44% C, 3.5% N, C-to-N ratio of 12.5) was equivalent to 1.5 mg C g^{-1} soil and 120 µg N g^{-1} soil. In the maize plant treatments, 4 seeds of maize were sown at a depth of 3 cm on 3 June 2002, two weeks after filling the soil into the pots, so that the decomposition of alfalfa residues in treatment 4 did not interfere with the germination of the plants. After emergence of the maize plants, two plants were screened out, so that each pot maintained two healthy plants up to maturity. At the beginning, moisture was maintained at 60% of the soil water holding capacity by weighing twice a week and adding the water lost regularly. The moisture was reduced to 40% water holding capacity after 8 weeks and then finally down to 30% after 10 weeks. The open bottom of each pot was covered by a net. The pots stood directly on flat dishes allowing aeration through the net-covered bottoms to a minor extent only.

Sampling of soil and plant material

Watering of the pots was stopped for 2 (with plants) or 7 d (without plants) before sampling. Sampling was done on four days replicate-wise (1^{st} day: one replicate of all treatments, 2^{nd}, 3^{rd} and 4^{th} day: two replicates each). In each pot, shoots of the maize plants above the soil surface and also the roots growing through the bottom of the pots

were removed, dried, weighed, and stored for further analysis. A soil sample of approximately 900 g was taken with a soil corer from the centre of each pot. After dry matter determination, the soil was sieved (< 2 mm) by carefully crumbling it between the fingers to remove maize roots and alfalfa residues. Soil adhering to roots and residues on top of the sieve were carefully removed from the roots and passed through the sieve. Separated maize roots and alfalfa residues were removed, dried, weighed, and stored for further analysis. The soil passed through the sieve was air-dried and stored for the analysis of particulate organic matter, soil organic C, total N and $\delta^{13}C$.

Soil microbial properties

Microbial biomass C, biomass N and biomass P were estimated by the fumigation-extraction method (Brookes et al., 1982; Brookes et al., 1985; Vance et al., 1987) using the pre-extraction procedure of Mueller et al. (1992) as modified by Mayer et al. (2003).

For pre-extraction, 4 x 60 g soil (for microbial biomass C and biomass N) and 6 x 5 g soil (for microbial biomass P) were transferred into 250 ml or 100 ml plastic bottles and 150 ml or 50 ml demineralised water were added, respectively. The bottles were shaken for 20 minutes at 200 rev. min^{-1}. The soil suspension was poured through a 2 mm sieve and 200 ml demineralised water were used to rinse roots and organic particles remaining on the sieve. Roots and organic particles were rejected. After stirring with a glass-stick, the suspension was allowed to sediment for at least 30 min. Roots and organic particles appearing at the surface of the suspension were removed using tweezers. The suspension was poured into a folded filter paper (Schleicher & Schuell 595½, Dassel, Germany; 240 mm diameter for 60 g soil and 150 mm for 5 g soil).

For estimating microbial biomass C and biomass N, two of the pre-extracted 60-g portions were fumigated for 24 h at 25°C with ethanol-free $CHCl_3$. In addition to the usual fumigation procedure, 3 drops of liquid $CHCl_3$ were added directly to the soil samples in the filter paper. The fumigants were removed before the soil, including the filter paper, was extracted with 200 ml 0.5 M K_2SO_4 by 30 min horizontal shaking at 200 rev min^{-1} and filtered (Schleicher & Schuell 595 ½). The non-fumigated portions plus filter paper were extracted similarly at the time fumigation commenced. Organic C in the extracts was measured as CO_2 by infra-red absorption after combustion at 850°C using a Dimatoc 100 automatic analyzer (Dimatec, Essen, Germany). Microbial biomass C was E_C / k_{EC}, where E_C = (organic C extracted from fumigated soil) – (organic C extracted from non-fumigated soil) and k_{EC} = 0.45 (Wu et al., 1990, Joergensen 1996). Total N in the extracts was measured as activated NO_2^* by chemoluminescence detection (Dima-N, Dimatec) after combustion at 850°C. Microbial biomass N was E_N / k_{EN}, where E_N = (total N extracted from fumigated soil) - (total N extracted from non-fumigated soil) and k_{EN} = 0.54 (Brookes et al., 1985; Joergensen and Mueller, 1996).

For estimating microbial biomass P, the first two 5-g portions were fumigated as described above, extracted with 100 ml 0.5 M $NaHCO_3$ (pH 8.5) by 30 min horizontal shaking at 200 rev min^{-1}, centrifuged for 15 min at (2000 *g*), and filtered (Schleicher & Schuell 595 ½). Two non-fumigated portions were extracted similarly at the time fumigation commenced. The remaining two portions were extracted after addition of 25 µg P g^{-1} (0.5 ml KH_2PO_4) in the same way as non-fumigated samples. Phosphate was measured by photo-spectrometry at 882 nm as described by Joergensen et al. (1995).

Ergosterol was measured in 2 g moist soil taken directly from the maize experiment. Ergosterol was extracted with 100 ml ethanol for 30 minutes by horizontal shaking at 250 rev min^{-1} and filtered (Whatman GF/A) (Djajakirana et al., 1996).

Quantitative determination was performed by reversed-phase HPLC analysis at 26°C using a column of 125 mm x 4 mm Sphereclone 5µ ODS II with a guard column of 4 mm x 3mm. The chromatography was performed with 100% methanol and a resolution of detection of 282 nm.

Evolved CO_2 was measured three times a week over a period of 2 minutes using a transportable infrared gas analyser with 2 to 5 replicates (Blanke, 1996). The dynamic system consisted of a chamber (100 mm diameter, 150 mm height) coupled to a portable infrared gas analyser (IRGA) in a closed circuit (PP Systems, Hitchin, Herts., UK). The flow rate through the IRGA sensor cell during measurements was approximately 0.5 l min^{-1}. Mixing of the air in the closed chamber during measurements was ensured by a small fan running at very low speed inside the chamber. Soil temperature at 5 cm depth was measured concurrently using an attached temperature probe. Periodical measurements were taken between 10 a.m. and 1 p.m. The difference between the average temperature of the day and the temperature during the CO_2 measurements was corrected using the rate-modifying factor proposed by Jenkinson et al. (1987):

$$y = 47.9 / [1 + e^{106 / (x + 18.3)}] \quad (1)$$

The CO_2 evolution rate data in mg CO_2-C m^{-2} h^{-1} were taken as representative for half of the period between two measuring points and temperature corrected according to the mean daily temperature during this interval. Finally, the CO_2 evolution rate was recalculated into µg CO_2-C g^{-1} soil for a specific interval or the whole experimental period.

Particulate organic matter and soil organic matter

Soil (400 g) was initially dispersed in 400 ml 5% NaCl, shaken by hand and allowed to stand for 45 min or over night. Then the samples were poured gradually onto sieves of 400 µm and subsequently 63 µm mesh sizes (Magid and Kjaergaard, 2001; Magid et al., 2004) and washed with tap water. The aggregates were destroyed by pushing the soil through the sieve during the washing procedure until the water passing through the sieve became clear. The material retained on the sieve was transferred into a beaker. Tap water was added, the bucket was swirled and organic material was separated from the mineral material by flotation-decantation. Swirling and flotation-decantation was repeated several times, until organic particles were no longer visible in the mineral fraction. Then, the mineral fraction was discarded. The remaining two fractions of particulate organic matter (POM) 63 – 400 µm and POM > 400µm were transferred to a crucible, dried at 60°C, and ground for further analysis.

Total C and total N were determined gas-chromatographically after combustion using a Vario Max CN analyser (Elementar, Hanau) and $\delta^{13}C$ was measured on a Delta plus IRMS 251 (Finnigan Mat, Bremen) after combustion using a Carlo Erba NA 1500 gas chromatograph. Maize root derived C in the POM fractions was calculated from the $\delta^{13}C$ data by a modified form of the equation used by Balesdent and Mariotti (1996) according to Mueller et al. (1998):

$$C_{dfm} = C_{affected} \times [(\delta^{13}C_{affected} - \delta^{13}C_{non\text{-}affected}) / (\delta^{13}C_{maize} - \delta^{13}C_{non\text{-}affected})] \quad (2)$$

where C_{dfm} is C derived from maize roots in the two POM fractions, $\delta^{13}C_{affected}$ and $\delta^{13}C_{non\text{-}affected}$ are $\delta^{13}C(PDB)[‰]$ in the two POM fractions from the maize root affected

or non-affected soil organic matter fractions, respectively. $\delta^{13}C_{maize}$ is $\delta^{13}C$(PDB)[‰] measured in the maize root material.

Results

Neither the addition of alfalfa residues, nor the growth of maize plants alone led to significant changes in any of the soil microbial properties analysed in comparison to the control soil (Table 1). In contrast to this, the combination of growing maize plants and alfalfa residues increased the contents, in most cases significantly, of microbial biomass C (36%), biomass N (28%), biomass P (15%) and ergosterol (24%). These differences in increases led to a significant increase in the microbial biomass C-to-N ratio, but the microbial biomass C-to-P ratio and the ergosterol-to-microbial biomass C ratio remained unchanged. The amount of the maize shoot C was more than doubled in treatment 4 with alfalfa residues than without in treatment 3 (Table 2). In these two maize growth treatments, the mean shoot C-to-N ratio was 90 and the mean shoot C-to-root C ratio was 5.3 without any significant treatment effects.

Plant remains > 2 mm increased significantly in the order alfalfa residues < maize roots < maize roots plus alfalfa residues (Table 3). Only 6% of the alfalfa residues could be recovered as plant remains > 2 mm in treatment 2. The two POM fractions 63–400 µm and 400–2000 µm comprised 10% and 4% of soil organic C. Only minor amounts of maize roots and alfalfa residues were transferred into these two fractions according to the non-significant differences between the three amendment treatments and the control. The differences were small in most cases, except treatment 4 with maize growth plus alfalfa residues. The $\delta^{13}C$ value of recovered alfalfa residues > 2 mm was lowest at -28.2 δ‰ and that of maize roots was highest at -14.9 δ‰. Based on these significantly

differing values, the contribution of these two sources to the fraction of plant remains > 2 mm could be calculated according to equation (2). Maize roots contributed 836 µg C g^{-1} soil to this fraction (Table 3) and alfalfa residues 144 µg C g^{-1} soil, equivalent to 85.3% and 14.7%, respectively. This means that 54 µg g^{-1} soil or 60% more alfalfa-C were recovered in treatment 4 than in treatment 2. The significantly higher $\delta^{13}C$ values in the POM fractions 63–400 µm and 400–2000 µm of treatment 4 in comparison to the control treatment revealed the occurrence of 63 µg maize root C g^{-1} soil in both fractions, equivalent to 4.6% and 14% of the two POM fractions.

In treatments 2 and 3, i.e. the sole alfalfa residues and sole maize growth treatments, the sum of CO_2-C evolved during the 90 day pot experiment were on the same level and nearly doubled in comparison to the control soil (Table 4). The sum of CO_2-C evolved in the maize plus alfalfa treatment was roughly 60% larger, i.e. the amount of substrate-derived CO_2-C of this treatment was twice that of the sole maize growth treatments. In treatment 2, the sum of substrate-derived CO_2-C was equivalent to 18% of the added alfalfa residue C. From 11 June (6 days after sowing) until 2 July, the CO_2 evolution rate of the two maize treatments was higher than that of both the sole alfalfa residues treatment and the control soil (Table 4; Fig. 2). From 3 July until the end of the experiment, the CO_2 evolution rate of the sole alfalfa residues treatment was only slightly above the control soil. Only 11% of the difference between these two treatments was produced during the last 48 days of the experiment (Table 4).

Table 1. Microbial biomass indices at the end of the experiment

	Microbial biomass C	Microbial biomass N	Microbial biomass P	Ergosterol	Microbial biomass C/N	Microbial biomass C/P	Ergosterol / microbial biomass C
	($\mu g\ g^{-1}$ soil)						(%)
Control	278 a	43 a	26 a	1.09 ab	6.5 a	12.9 a	0.40 ab
Alfalfa residues	276 a	44 a	29 a	1.20 ab	6.3 a	11.3 a	0.44 b
Maize	304 a	46 a	38 a	1.01 a	6.6 ab	7.9 a	0.34 a
Maize + alfalfa residues	378 b	55 b	37 a	1.35 b	6.9 b	10.4 a	0.36 ab

Different letters within a column indicate a significant difference ($P < 0.05$, Tukey/Kramer, n = 7)

Table 2. Organic C in shoot and root material > 2 mm, ratios shoot organic C-to-total N and shoot C-to-root C at the end of the experiment

	Shoot C (g pot^{-1})	Shoot C/N	Root C (g pot^{-1})	Shoot C/root C
Maize	20.8 a	94 a	4.2 a	5.4 a
Maize + alfalfa residues	45.5 b	86 a	9.0 b	5.2 a

Different letters within a column indicate a significant difference ($P < 0.05$, Tukey/Kramer, n = 7)

Table 3. Organic C content and $\delta^{13}C$ values in plant remains > 2 mm and in the particulate organic matter (POM) fractions 63–400 µm and 400–2000 µm at the end of the experiment, in brackets maize root-derived organic C according to the differences in $\delta^{13}C$ values calculated with equation (2)

	POM-C 63–400 µm	POM-C 400–2000 µm	Plant remains > 2 mm
Organic C (µg g^{-1} soil)			
Control	1050 a	390 a	ND
Alfalfa residues	1100 a	390 a	90 a
Maize	1010 a	410 a	460 b
Maize + alfalfa residues	1400 a (63)	450 a (63)	980 c (836)
$\delta^{13}C$ (‰)			
Control	-27.9 b	-27.8 c	ND
Alfalfa residues	-27.4 a	-26.7 ab	-28.2 c
Maize	-27.7 ab	-27.7 bc	-14.9 a
Maize + alfalfa residues	-27.3 a	-26.0 a	-16.8 b

Different letters within a column of a specific block indicate a significant difference ($P < 0.05$, Tukey/Kramer, n = 7)

Table 4. Sum of CO_2-C evolved and substrate-derived sum of CO_2-C (substrate treatment minus control) during the complete 90-day greenhouse experiment and during 4 different sub-periods of roughly three weeks

	CO_2-C 05/22–06/10 20 d ($\mu g\ g^{-1}$ soil)	CO_2-C 06/11–07/02 22 d ($\mu g\ g^{-1}$ soil)	CO_2-C 07/03–07/25 23 d ($\mu g\ g^{-1}$ soil)	CO_2-C 07/26–08/19 25 d ($\mu g\ g^{-1}$ soil)	ΣCO_2-C 05/22–08/19 90 d ($\mu g\ g^{-1}$ soil)
Control	68 a	92 a	133 a	112 a	406 a
Alfalfa residues	224 b	182 b	158 a	118 a	682 b
Maize	62 a	161 b	275 b	271 b	769 b
Maize + alfalfa residues	192 b	293 c	283 b	375 c	1143 c
Treatment – control					
Alfalfa residues	156	90	25	6	276
Maize	-6	69	142	159	363
Maize + alfalfa residues	124	201	150	263	737

Different letters within a column indicate a significant difference ($P < 0.05$, Tukey/Kramer, n = 7)

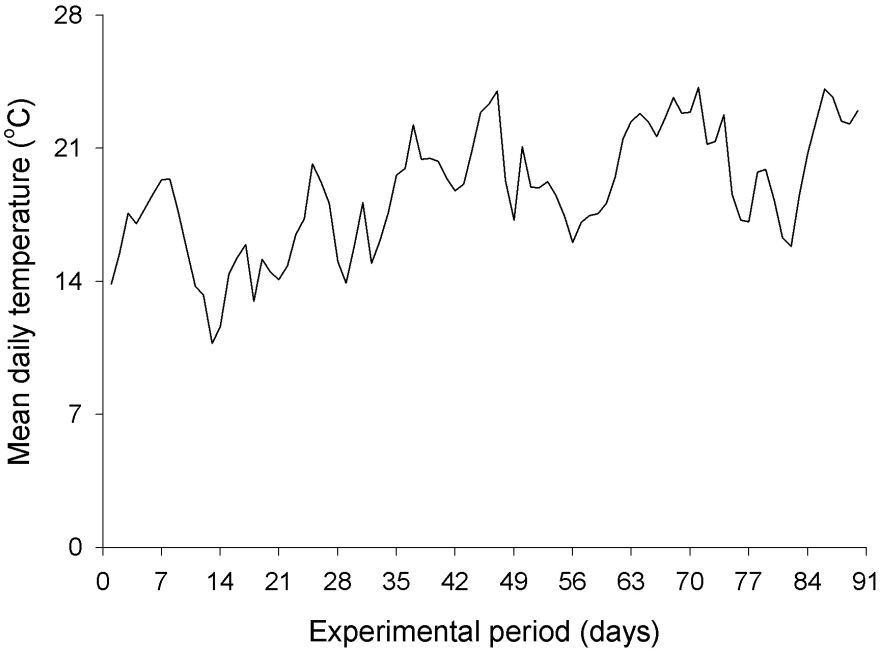

Figure 1. Mean daily temperature in the greenhouse during the experiment from 22 May to 19 August 2001

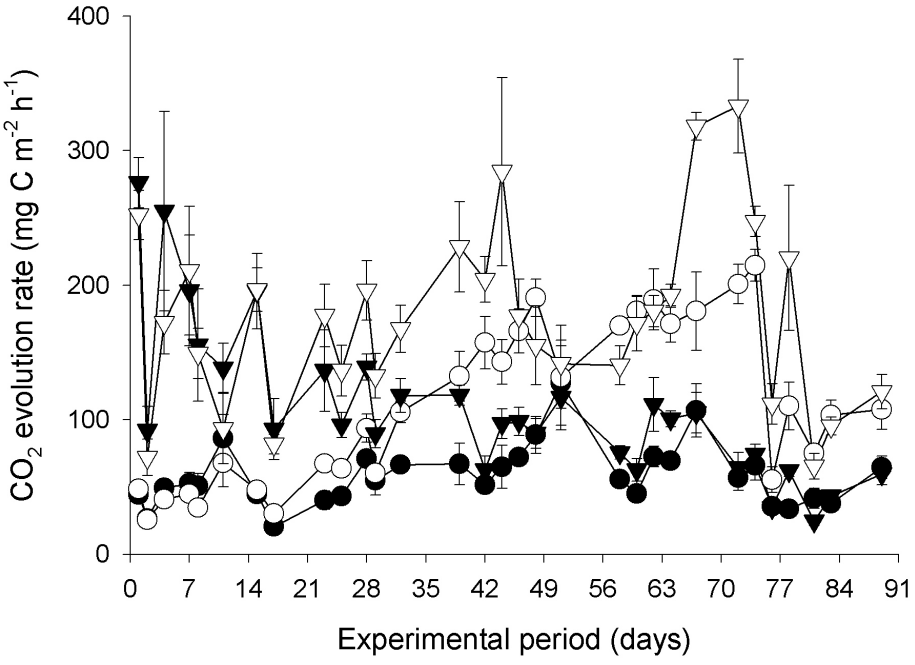

Figure 2. CO_2 evolution rates in the different treatments in the greenhouse during the experiment from 22 May to 19 August 2001; control (●), alfalfa residues (▼), maize (○), and maize + alfalfa residues (▽)

Discussion

The sole effects of alfalfa residues and maize plant growth on soil microbial properties are negligible, although both treatments add considerable amounts of easily available substrate to soil, increasing the microbial turnover as indicated by significantly increased CO_2 evolution treatments. This constancy supports the hypothesis of a biological space in soil maintaining very stable amounts of microbial biomass (Nannipieri et al., 1978). In accordance with this, Chen et al. (2003) did not measure an increase in substrate-induced respiration at the end of a 56-day micro-pot experiment with growing wheat plants after the amendment of 20 mg alfalfa g^{-1} soil, but they measured a strong increase in microbial biomass N, indicating a method-specific reaction on the experimental conditions. In the maize plus alfalfa residues treatment of the present experiment, the four soil microbial indices varied in a considerably smaller range and increased on average by roughly 30% in comparison to the sole maize treatment, without indication of a serious change in the microbial community on the basis of the nearly constant gross ratios microbial biomass C-to-N, microbial biomass C-to-P ratio, and ergosterol-to-microbial biomass C.

In contrast to the microbial biomass indices, the amount of shoot C and root C, i.e. the C input into soil showed not only a 30% increase, but revealed more than two times larger yields in the maize plus alfalfa residues treatment in comparison to the sole maize treatment. It can be assumed that this increase in maize growth by the addition of alfalfa was mainly due to the release of nutrients during decomposition. However, the C-to-N ratio in the shoot biomass did not differ in the two maize plant treatments, indicating that N was not the only limiting nutrient for maize growth. Also the ratio shoot C-to-root C was identical in the two maize growth treatments of the present pot experiment, but this ratio was apparently shifted towards shoot production in comparison to the field

situation, probably due to sufficient water supply (Richner et al., 1996; Liedgens and Richner, 2001).

Only 6% and 10% of the alfalfa residues added were recovered as particulate organic matter > 2 mm, indicating nearly complete decomposition during the experiment. This fast turnover is in contrast to many other studies undertaken using the litter-bag approach. After two years of field incubation in nylon nets, the remaining alfalfa C of a mixture with a red-earth extremely low in organic matter varied in a depth-specific way between 28 and 19% (Rovira and Vallejo, 2000). In this nearly C-free soil material, a certain part of the added organic material might have been used to build up soil organic matter. In an incubation experiment at constant 20ºC, roughly 60% of alfalfa residues disappeared from litter-bags placed on the soil surface within 40 days (Dalias et al., 2003). However, some studies reporting faster turnover of plant residues even in litter bags support our findings. Bross et al. (1995) carried out a field experiment with litter-bags and measured a decrease in alfalfa residues between 90% and 96% during summer in Michigan, USA. Annual decomposition rate coefficients (-r, $C_{t1} = e^{-r} C_{t0}$, C = amount of C, t1 = 1 year) of 4.61 and 5.12 could be measured in the present sole alfalfa residues treatment and in the maize plus alfalfa residues treatment, respectively. This is consistent with Schomberg and Steiner (1999), who measured a decomposition rate coefficient of 4.4 in a field experiment. Consequently, mass losses of 94% and 90% are possible for alfalfa residues under the environmental conditions of the present pot experiment.

In the laboratory, 58% of added alfalfa C was mineralised as CO_2 within 30 days' incubation at 25ºC (Ajwa and Tabatabai, 1994). Magid and Kjaergaard (2001) and Magid et al. (2004) observed sufficient agreement between the residues left in the soil and that part mineralised as CO_2. However, the large loss rates of alfalfa residues in our experiment contrast the CO_2 evolution rates observed: Only 18% of the alfalfa residues

were measured as CO_2. Consequently, it can be ruled out that the addition of alfalfa residues enhanced the decomposition of native soil organic matter considerably. More likely would be a reduction in the decomposition of soil organic matter after the addition of alfalfa residues, which was defined as a negative priming effect (Kuzyakov et al., 2000). However, only positive priming effects, i.e. a stimulation e.g. in the mineralization of native soil organic N was reported in the literature after the addition of alfalfa residues (Pare and Gregorich, 1999).

An unknown amount of the CO_2 evolved might be lost through the net-covered opening at the bottom of the pots, but this must have been true for the control treatment too. Nearly 4% of soil organic C was mineralised in the control treatment, i.e. a very large percentage within 3 months, indicating that the general level of the CO_2 evolvement cannot have been underestimated considerably. Two factors contribute presumably significantly to the strong underestimation of alfalfa residues by the CO2 evolution rate: (1) Peak concentrations in CO_2 were not appropriately measured, especially in the early decomposition period and (2) the rate modifying factor of Jenkinson et al. (1987) is invalid for an easily decomposable material such as alfalfa residues leading to underestimations in the CO_2 evolution rates at lower temperatures (Cookson et al. 2002).

An interesting feature of our experiment is the apparent reduction in the decomposition of alfalfa residues in the maize plus alfalfa residues treatment. The difference between 6 and 10% alfalfa residues left in the soil is not extremely large, but it is supported by the CO_2 evolution rate. Assuming a constant ratio of root C to root respiration, the root- and alfalfa residues-derived amount of ΣCO_2 should be 363 / 4.3 x 9.0 + 276 = 1036 µg ΣCO_2-C g^{-1} soil or 41% larger (see Tables 2 and 4), indicating a reduced specific root respiration or a reduced mineralization of alfalfa residues or both. The three-week period from 11 June to 2 July should have been the main period when

interaction between decomposition and root growth could occur. Before, no significant root respiration was observed, after the 2 July only minor percentages of the alfalfa residues added were further decomposed (Table 4, Fig. 2). However, the largest relative retardation of CO_2 evolution was observed between 2 July and 25 July. The reasons cannot be fully explained; deficiency in oxygen and water demand may contribute to the observed relative retardation. Faber and Verhoeff (1991) found a significant reduction in the decomposition of fragmented litter by black pine (*Pinus nigra*) roots. They explained their observation by a reduced N availability to soil microorganisms, an unlikely reason in the present experiment with N rich alfalfa litter. A relative decline of microbial respiration with increasing ratio of substrate to soil microbial biomass was repeatedly observed for compost (Niklasch and Joergensen, 2001) and straw (Fruit et al., 1999). This relationship might have contributed to the present retardation in the decomposition of alfalfa litter.

Conclusions

The recovery of non-decomposed residues by sieving in combination with differences in $\delta^{13}C$ signature between C3 and C4 plants gives the opportunity to analyse the interactions between plant growth and residue decomposition. The original hypotheses, assuming an increased turnover of alfalfa residues in the presence of living roots, must be negated at least under the conditions of this study. However, the explanatory power of the present approach was limited by the rapid decomposition of alfalfa residues in relation to the development of maize plants.

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Chapter 3

Relationships between soil biological and other soil properties in saline and alkaline arable soils from the Pakistani Punjab

Relationships between soil biological and other soil properties in saline and alkaline arable soils from the Pakistani Punjab

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Abstract

The interactions between soil physical, soil chemical and soil biological properties were analysed in 30 Pakistani soils from alkaline and saline arable sites differing strongly in salinisation and in soil pH. The soil biological properties were differentiated into indices for microbial activity (basal respiration), microbial biomass (C, N, and P), and community structure (fungal ergosterol) with the aim of assessing their potential as soil fertility indices in alkaline and saline arable soils. All soils were salt affected, mainly by Na^+ and HCO_3^-, and their texture was dominated by silt. The median contents of soil organic C, total N and total P were 4.5 mg g^{-1} soil, 0.45 mg g^{-1} soil, and 0.66 mg g^{-1} soil, respectively. On average, only 1.0% of total P was extractable by 0.5 M $NaHCO_3$. The median contents of microbial biomass C, biomass N, biomass P, and ergosterol were 94 µg g^{-1} soil, 11 µg g^{-1} soil, 4.6 µg g^{-1} soil, and 0.16 µg g^{-1} soil, respectively. Basal respiration varied around a median of 18.0 µg CO_2-C d^{-1} g^{-1} soil and was the only soil biological property negatively affected by increasing salinity. Ergosterol was negatively related to soil pH. The microbial biomass C-to-P ratio exceeded the soil organic C-to-total P ratio nearly threefold, but the soil organic C-to-total N ratio and microbial biomass C-to-N ratio were both at a similar level around 9.0. For this reason, the microbial biomass C-to-soil organic C ratio and the microbial biomass N-to-total N ratio were also both at a similar level at approximately 2.2%. The microbial biomass P-total P ratio was three times smaller. Salt-derived Na also had some negative effects on the metabolic quotient qCO_2. High levels of the metabolic quotient qCO_2 indicate that the microorganisms are generally stressed by the saline and alkaline conditions, leading to low substrate use efficiencies. This intensifies the threat of further losses in soil fertility by the reduction in soil organic matter levels.

Keywords: Sodium • Bicarbonate • Microbial biomass • Ergosterol • CO_2 evolution

1. Introduction

The assessment of soil fertility, i.e. the ability of a soil to provide nutrients for plant growth to gain a certain crop yield and the assessment of soil quality, i.e. the capacity of a soil to maintain key ecological functions such as decomposition and formation of organic matter, are important objectives in tropical and subtropical regions (Srivastava and Lal, 1994; Wick et al., 1998; Joergensen and Castillo, 2001). Since temperature controls many processes in soil, especially microbiologically mediated ones, the higher temperatures in tropical and subtropical regions lead to faster turnover rates of microbial biomass and soil organic matter in comparison to temperate Northern Europe, shortening the time taken for ecosystems to respond to changes in management practices (Grisi et al., 1998). In arid or semi-arid subtropical regions such as Arabia or Pakistan, temperature effects on soil microorganisms are often counteracted by strong and rapid changes in soil moisture content, due to dry and rainy seasons or irrigation management considerably affecting soil biological processes (Wichern et al., 2004).

The effects of extreme seasonal variations in water content have been investigated in highly weathered soils from old land surfaces in Africa (Wick et al., 1998), in soils from Gangetic alluvium in India (Srivastava and Lal. 1994), and from volcanic ash soils in Costa Rica and Nicaragua (Mazzarino et al., 1993; Joergensen and Castillo, 2001). No information is available on the biological properties of soils from arid and semi-arid sub-tropical regions of Pakistan, where salinity is usually combined with high pH conditions, due to the presence and enrichment of calcium carbonate in the uppermost soil layers (alkaline saline soils) or to hydrolysis of sodium carbonate (sodic soils). In

these regions, shallow water tables and improper irrigation practices often accelerate salinisation. Consequently, salinity affects 30% of arable land and is a major threat to plant growth (Sandhu and Qureshi 1986; Qureshi and Barret-Lenard 1998). Monovalent cations such as sodium play a very important role in deflocculating clay particles and consequently in increasing the tendency to slake, disperse and swell under specific conditions, so that soils become impermeable and ultimately infiltration and percolation of water cease from the upper surface and within the soils (Keren 2000; Qadir and Schubert 2002). Sardinha et al. (2003) analysed soils from a salinity gradient under acidic conditions and concluded that salinisation is one of the most stressing environmental conditions for soil microorganisms, especially for fungi.

The central objective of the present research was to analyse the interactions between the most important soil physical (texture), soil chemical (pH, salinity indices, soil organic matter, P status) and soil biological properties in Pakistani soils, investigating 30 typical alkaline and saline arable sites differing strongly in salinisation and in soil pH. The soil biological properties were differentiated into indices for microbial activity (basal respiration), microbial biomass (C, N, and P), and community structure (fungal ergosterol), with the aim of assessing their potential as soil fertility and soil quality indices in alkaline and saline arable soils. The hypotheses were: (1) Increasing salinity and increasing alkalinity both have depressive effects on microbial biomass and activity indices and (2) increasing salinity and increasing alkalinity both lead to strong changes in microbial community structure towards bacteria.

2. Material and methods

2.1. Soil sampling and specification

Soils included in the present study were collected from 30 arable sites of the province Punjab (33.36°N and 73.07°E), central Pakistan, in the regions of Faisalabad, Lahore, Sargodha, Gujranwala, Gujrat, and Rawalpindi, forming a gradient in annual precipitation (Table 1). The local climate is characterised by two distinct seasons, a very hot summer from June to August with maximum mean monthly temperatures up to 49°C and a cool period from October to February with minimum mean monthly temperatures down to 2.6°C. The mean annual precipitation is unevenly distributed over the year, i.e. approximately 50% comes in July. Thirty sites were selected and four independent samples were taken from each site in July 2002. The soils were sampled at 0-15 cm depth with a soil corer (4 x 15 cm), sieved (< 2 mm) and transported to Witzenhausen, Germany.

2.2. Soil physical and chemical properties

Soil textural analysis was carried out after treatment of 30 g soil with 7% H_2O_2 for two weeks, removal of carbonate with 10% HCl, suspension in $NaPO_4$, sieving through a sieve with 63 µm diameter and weighing of the sand fraction. The remaining sample <63 µm was processed further for clay and silt determination by a pipette method (Schlichting et al., 1995). Soil pH was measured using a soil-to-water 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:

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

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. Sub-samples of dried soil material were homogenised in a ball mill. Total C and total N were determined using a Vario Max CN analyser (Elementar, Hanau, Germany). Soil organic C was measured as total C minus carbonate C, which was measured gas-volumetrically after the addition of 10% HCl (Schlichting et al., 1995). Total P was 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).

Amount and composition of soluble salts were determined after suspending 5 g soil in 50 ml demin. water for 1 h and centrifugation at 2000 g. The concentrations of Na^+, K^+, Mg^{2+}, and Ca^{2+} were determined by atomic absorption spectrometry, the concentrations of HCO_3^-, SO_4^{2-} and Cl^- potentiometrically. The sodium adsorption ratio (SAR) was calculated as:

$$\text{SAR} = \frac{[Na^+]}{\sqrt{0.5([Ca^{2+}]+[Mg^{2+}])}}$$

Cation exchange capacity was determined according to Ryan et al. (1996). A 4 g soil sample was extracted wit 33 ml of a 1 M sodium acetate solution for 5 min, centrifuged at 1000 *g* until the supernatant solution was clear. The supernatant was decanted as completely as possible and discarded. This process was repeated 4 times and then, finally, the sample was extracted with 33 ml of 95% ethanol for 5 min, and centrifuged at 1000 *g* until the supernatant solution was clear. The sample was washed with 33 ml portions of ethanol until the EC of the supernatant from the previous time was less than

400 µS cm^{-1}. The exchangeable Na was extracted from the sample with three 33 ml portions of 1 M ammonium acetate solution for 5 min, centrifuged at 1000 *g* until the supernatant solution was clear. Finally, sodium in the combined ammonium acetate solutions was determined by atomic absorption spectrometry.

2.3. Soil microbial properties

For the measurement of basal respiration, 100 g soil on an oven-dry basis was weighed into a 1 l Pyrex jar, adjusted to 50% of its maximum water holding capacity and incubated for 7 days at 25°C in the dark. Soil respiration was continuously measured as O_2 consumption using a Sensomat (Aqualytic, Darmstadt, Germany) tension-recording device (Robertz et al., 1999). The CO_2 produced was absorbed in 40% KOH. For better comparison with published data, the CO_2 production was then calculated from the O_2 consumption, assuming a respiratory quotient of 1. However, it should be noted that this assumption is true for the decomposition of glucose-like substrates only and might be wrong for the soil organic matter in true soil samples like those of this study (Dilly, 2001). The metabolic quotient qCO_2 was calculated as follows: (µg CO_2-C g^{-1} soil evolved during 7 days) / (µg microbial biomass C g^{-1} soil) / 7 days x 1000 = mg CO_2-C d^{-1} g^{-1} biomass C.

For estimating microbial biomass C (Vance et al., 1987) and biomass N (Brookes et al., 1985) by fumigation-extraction, two portions equivalent to 25 g oven dry soil were taken from the 100-g soil sample at the end of the 7-day incubation period for measuring basal respiration. One portion was fumigated for 24 h at 25°C with ethanol-free $CHCl_3$. The fumigant was removed before 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 through a folded filter

paper (Schleicher & Schuell 595 ½, Dassel, Germany). 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 E_C / k_{EC}, where E_C = (organic C extracted from fumigated soil) – (organic C extracted from non-fumigated soil) and k_{EC} = 0.45 (Wu et al., 1990, Joergensen 1996). Total N in the extracts was measured as activated NO_2^* by chemoluminescence detection (Dima-N, Dimatec) after combustion at 850°C. Microbial biomass N was E_N / k_{EN}, where E_N = (total N extracted from fumigated soil) - (total N extracted from non-fumigated soil) and k_{EN} = 0.54 (Brookes et al., 1985; Joergensen and Mueller, 1996).

For estimating microbial biomass P (Brookes et al., 1982) by fumigation-extraction, three portions equivalent to 2.5 g oven dry soil were taken from the 100-g soil sample at the end of the 7-day incubation period. The first 2.5-g portion was fumigated as described above, extracted with 50 ml 0.5 M $NaHCO_3$ (pH 8.5) by 30 min horizontal shaking at 200 rev min^{-1}, centrifuged for 15 min at (2000 *g*), and filtered (Schleicher & Schuell 595 ½). One non-fumigated portion was extracted similarly at the time fumigation commenced. The remaining portion was extracted after addition of 25 µg P g^{-1} (0.5 ml KH_2PO_4) in the same way as non-fumigated samples. Phosphate was measured by photo-spectrometry at 882 nm as described by Joergensen et al. (1995).

Ergosterol was measured in 2 g moist soil taken from the 100-g soil sample at the end of the 7-day incubation period. Ergosterol was extracted with 100 ml ethanol for 30 minutes by horizontal shaking at 250 rev min^{-1} and filtered (Whatman GF/A) (Djajakirana et al., 1996). Quantitative determination was performed by reversed-phase HPLC analysis at 26°C using a column of 125 mm x 4 mm Sphereclone 5µ ODS II with a guard column of 4 mm x 3mm. The chromatography was performed with 100% methanol and a resolution of detection of 282 nm.

2.4. Statistics

The results presented in the tables are arithmetic means and expressed on an oven-dry basis (about 24 h at 105 °C). The relationships between the different soil properties were analysed by principal component analysis (PCA) using the orthotran/varimax rotation to achieve either small or large component loading and an Eigenvalue of 0.9 as the lower limit. All statistical analyses were performed using StatView 5.0 (SAS Inst. Inc.).

3. Results

The texture of the 30 arable soils investigated was dominated by the silt fraction, with a median of 59%, followed by the most variable sand fraction ranging from 4 to 82%, with a median of 26%, and the clay fraction, with a median of 15% (Table 1). Also the cation exchange capacity varied strongly around a median of 130 $\mu mol_C\ g^{-1}$ soil. All soils were in the alkaline range, with a median $CaCO_3$ content of 4.7% and a soil pH varying between 8.1 and 10.4. All soils were salt affected and contained between 1.3 and 36.1 mg salt g^{-1} soil, corresponding to EC values of between 2.1 and 62.9 mS cm^{-1} (Table 3). The median of the sodium absorption ratio was 8.3; the levels of the different values were not directly related to differences in the salt concentration, but to strong differences in the salt composition between the different soils (Table 4). The cation composition was dominated by Na, with a median of 45.7 $\mu mol_C\ g^{-1}$ soil or 89% of the cation sum (Table 4), followed on a similar, but very low level by Mg, Ca, and K, with medians of 2.1, 2.0 and 1.6 $\mu mol_C\ g^{-1}$ soil, respectively, corresponding to 4%, 4% and 3% of the cation sum. The anion composition was dominated by

bicarbonate, with a median of 23.0 $\mu mol_C\ g^{-1}$ soil, followed by sulphate and chloride, with medians of 17.6 and 8.8 $\mu mol_C\ g^{-1}$ soil, respectively, corresponding to 46%, 36% and 18% of the anion sum.

The median contents of soil organic C, total N and total P were 4.5 mg g^{-1} soil, 0.45 mg g^{-1} soil, and 0.66 mg g^{-1} soil, respectively (Table 5). Total P was the soil property with the lowest soil-to-soil variation and exceeded total N by nearly 50%. On average, 1.2% of soil organic C was extractable with 0.5 M K_2SO_4 and 1.0% of total P was extractable by 0.5 M $NaHCO_3$. The median contents of microbial biomass C, biomass N and biomass P were 94 µg g^{-1} soil, 11 µg g^{-1} soil, and 4.6 µg g^{-1} soil, respectively (Table 6). As total P, microbial biomass P was the soil biological property with the lowest soil-to-soil variation. In contrast, the fungal biomarker ergosterol was highly variable, exhibiting a range from 0.01 to 1.00 µg g^{-1} soil, with a median of 0.16 µg g^{-1} soil. Basal respiration varied considerably less around a median of 8.6 µg CO_2-C d^{-1} g^{-1} soil.

Soil organic matter and microbial biomass indices formed the dominating factor 1 of the principal component analysis, characterising the biological space of the soil (Table 7). Salt-derived Na (but also any other of the various salinisation indices – results not shown) did not have any influence on factor 1. Only the respiration rate was negatively affected with increasing content of salt-derived Na, forming together factor 2, characterising salinity effects. Although total P and microbial biomass P both exhibited small soil-to-soil variations, these two properties could not be assigned to the same factor, but were separated into factor 3, characterizing the variation of total P, and factor 4, characterising the storage of P in the microbial biomass. Ergosterol was negatively combined with soil pH to form factor 5, characterising changes in the community structure of alkaline soils.

Table 1
Climatic conditions of 30 different arable sites used for sampling of Pakistani soils, soil type assignation according to the FAO classification system

Soil	*Area*	*Temperature (°C)*		*Precipitation*	*Soil type*
		Min	Max	(mm)	
01	Faisalabad	5.0	45.5	372	Haplic solonetz
02	Faisalabad	5.0	45.5	372	Haplic solonetz
03	Faisalabad	5.0	45.5	372	Haplic solonetz
04	Faisalabad	5.0	45.5	372	Salic solonetz
05	Lahore	5.9	48.0	536	Haplic solonetz
06	Lahore	5.9	48.0	536	Salic solonetz
07	Lahore	5.9	48.0	536	Salic solonetz
08	Lahore	5.9	48.0	536	Haplic solonetz
09	Lahore	5.9	48.0	536	Haplic solonetz
10	Lahore	5.9	48.0	536	Salic solonetz
11	Sargodha	3.6	49.0	611	Haplic solonetz
12	Sargodha	3.6	49.0	611	Haplic solonetz
13	Sargodha	3.6	49.0	611	Salic solonetz
14	Sargodha	3.6	49.0	611	Haplic solonetz
15	Sargodha	3.6	49.0	611	Salic solonetz
16	Sargodha	3.6	49.0	611	Salic solonetz
17	Gujranwala	5.0	45.5	720	Haplic solonetz
18	Gujranwala	5.0	45.5	720	Haplic solonetz
19	Gujranwala	5.0	45.5	720	Haplic solonetz
20	Gujranwala	5.0	45.5	720	Haplic solonetz
21	Gujranwala	5.0	45.5	720	Haplic solonetz
22	Gujranwala	5.0	45.5	720	Haplic solonetz
23	Gujranwala	5.0	45.5	720	Haplic solonetz
24	Gujranwala	5.0	45.5	720	Haplic solonetz
25	Gujranwala	5.0	45.5	720	Haplic solonetz
26	Gujranwala	5.0	45.5	720	Salic solonetz
27	Gujranwala	5.0	45.5	720	Salic solonetz
28	Gujranwala	5.0	45.5	720	Salic solonetz
29	Gujrat	5.1	43.0	747	Haplic solonetz
30	Rawalpindi	2.6	38.7	1178	Haplic solonetz

Table 2
Physical and chemical properties of soils from 30 Pakistani arable sites

	Soil Clay (%)	Silt (%)	Sand (%)	$CaCO_3$ (%)	pH-H_2O	CEC ($\mu mol_C\ g^{-1}$ soil)
01	14	48	38	2.6	9.2	118
02	18	48	34	4.2	9.0	174
03	16	65	9	5.2	9.7	153
04	14	61	25	7.2	8.5	190
05	16	75	9	4.1	9.3	169
06	20	57	23	2.0	8.9	147
07	**22**	66	12	2.4	9.4	162
08	14	72	14	2.6	9.4	146
09	16	**72**	12	1.8	9.9	183
10	12	50	38	1.6	10.2	135
11	10	45	45	8.4	9.2	33
12	15	46	39	10.5	9.6	46
13	12	64	24	13.0	9.7	45
14	13	69	18	10.0	**10.4**	**17**
15	13	73	14	7.8	8.4	117
16	16	74	10	9.9	**8.1**	118
17	**6**	**12**	**82**	**0.0**	8.4	72
18	8	26	66	2.7	8.9	77
19	16	30	54	2.9	9.8	104
20	13	69	18	1.2	9.6	**222**
21	18	38	44	12.5	8.9	127
22	15	46	39	10.0	8.7	75
23	14	58	28	8.5	9.1	44
24	16	54	30	7.6	9.2	49
25	13	32	55	3.2	9.9	70
26	12	62	26	4.4	9.3	150
27	10	65	25	16.1	8.2	161
28	17	65	18	7.4	8.6	216
29	17	54	29	1.7	9.0	152
30	16	**80**	**4**	**19.2**	9.2	158
CV (±%)	13	6	8	7.6	2.1	9

CV = mean coefficient of variation between replicate sites (n = 4), bold: minima and maxima

Table 3
Salt concentration, electrical conductivity (EC) and sodium absorption ratio (SAR) of soils from 30 Pakistani arable sites

Soil	Salt (mg g^{-1} soil)	EC (mS cm^{-1})	SAR
01	2.0	3.4	2.5
02	3.6	8.3	4.6
03	6.0	10.9	12.7
04	7.4	22.4	8.7
05	4.5	9.8	20.4
06	4.5	15.3	13.7
07	5.1	15.4	19.4
08	5.5	8.4	16.3
09	6.0	9.1	12.2
10	9.0	17.8	21.3
11	1.9	2.6	2.1
12	4.2	5.2	12.0
13	5.4	17.1	21.4
14	10.5	2.1	**112.1**
15	17.4	45.0	21.2
16	22.2	31.4	7.9
17	**1.3**	**2.1**	**1.2**
18	1.5	3.0	1.4
19	2.1	3.1	4.4
20	2.1	3.8	3.3
21	2.1	5.7	2.7
22	2.3	5.1	2.1
23	2.8	5.7	4.5
24	3.1	8.6	6.7
25	3.3	5.7	6.7
26	4.0	13.1	11.0
27	6.0	16.0	8.5
28	**36.1**	**62.9**	23.5
29	3.1	6.8	6.2
30	4.2	10.9	22.4
CV (±%)	1.6	2.5	1.2

CV = mean coefficient of variation between replicate sites (n = 4), bold: minima and maxima

Table 4
Cations and anions in the salt washed out from soils of 30 Pakistani arable sites

Soil	Na	K	Mg	Ca	HCO_3	Cl	SO_4
				($\mu g\ g^{-1}$ soil)			
01	280	103	51	109	1250	175	69
02	670	203	79	255	1460	239	654
03	1680	250	128	68	1720	489	1676
04	1580	190	84	478	900	1077	3114
05	1370	19	23	31	1520	587	993
06	1350	60	46	71	1430	552	1015
07	1560	43	33	46	1520	546	1327
08	1610	98	54	64	2040	543	1051
09	1710	**266**	128	85	2520	378	913
10	2810	156	130	46	2260	1112	2483
11	180	77	23	62	1480	69	29
12	1030	115	51	26	1990	729	269
13	1480	120	60	18	2100	1028	569
14	3260	79	56	**0**	**3480**	2122	1512
15	4780	54	74	629	1240	2520	8083
16	3650	66	**206**	**2875**	830	**3945**	10599
17	**120**	**0**	17	106	970	43	49
18	130	19	36	101	1050	**31**	27
19	410	81	61	33	1280	55	141
20	330	9	33	94	1500	34	41
21	310	65	44	126	1300	78	202
22	240	78	36	159	1070	54	671
23	480	43	43	103	1480	107	580
24	640	18	36	76	1230	159	912
25	630	218	83	**0**	1540	143	691
26	980	24	36	66	1340	225	1318
27	1450	8	62	336	950	1057	2093
28	**9350**	30	131	2138	**680**	3296	**20517**
29	750	213	88	78	1890	123	**4**
30	1210	**0**	**5**	43	1400	339	1175
CV (±%)	28	44	27	29	11	39	62

CV = mean coefficient of variation between replicate sites (n = 4), bold: minima and maxima

Table 5
Contents of soil organic C, 0.5 M K_2SO_4 extractable C, total N, total P, 0.5 M $NaHCO_3$ (pH 8.5) extractable P of soils from 30 Pakistani arable sites

Soil	Soil organic C (mg g^{-1} soil)	K_2SO_4 extractable C (µg g^{-1} soil)	Total N (mg g^{-1} soil)	Total P (mg g^{-1} soil)	$NaHCO_3$ extractable P (µg g^{-1} soil)
01	3.9	60	0.40	0.64	3.4
02	7.2	77	0.70	0.76	6.5
03	4.6	51	0.49	0.76	9.8
04	5.4	67	0.60	0.87	7.7
05	3.5	39	0.66	0.62	8.2
06	4.9	55	0.74	0.59	5.9
07	5.0	63	0.74	0.62	5.1
08	**7.5**	**99**	**0.92**	0.65	11.3
09	3.9	51	0.59	0.66	10.9
10	2.2	79	0.38	0.54	**11.4**
11	3.8	51	0.40	0.61	6.6
12	3.2	51	0.39	0.61	7.3
13	5.5	65	0.41	0.60	6.1
14	3.5	27	0.29	0.71	8.2
15	6.8	59	0.77	0.78	5.6
16	5.5	53	0.45	0.68	8.0
17	4.3	80	0.41	0.56	10.6
18	2.6	40	0.37	0.57	8.1
19	3.8	41	0.34	0.57	5.6
20	5.1	46	0.74	**0.51**	6.9
21	6.8	62	0.71	0.71	8.3
22	4.5	44	0.37	0.73	5.7
23	6.6	66	0.57	0.81	7.9
24	4.6	47	0.43	0.78	5.2
25	**1.9**	24	**0.23**	**0.88**	10.6
26	3.7	44	0.39	0.75	4.8
27	5.4	42	0.55	0.67	3.9
28	2.9	52	0.42	0.56	8.7
29	3.6	64	0.49	0.53	7.0
30	3.3	**21**	0.24	0.67	**2.7**
CV (±%)	13	17	9.8	3.1	35

CV = mean coefficient of variation between replicate sites (n = 4), bold: minima and maxima

Table 6
Microbial biomass C, biomass N, biomass P, ergosterol and CO_2 evolution rate in soils from 30 Pakistani arable sites

Soil	Microbial biomass C (µg g^{-1} soil)	Microbial biomass N (µg g^{-1} soil)	Microbial biomass P (µg g^{-1} soil)	Ergosterol (µg g^{-1} soil)	CO_2-C (µg C d^{-1} g^{-1} soil)
01	87	13.4	4.5	0.20	27.2
02	131	13.7	**5.9**	0.23	21.4
03	127	10.4	4.4	0.05	11.1
04	118	14.4	5.1	0.15	21.4
05	68	8.3	5.2	0.11	13.8
06	153	11.4	4.3	0.16	19.6
07	101	8.5	4.3	0.21	17.6
08	208	18.4	4.3	0.24	24.4
09	88	7.2	5.3	0.08	18.0
10	43	8.5	4.5	0.06	11.6
11	108	15.2	5.1	0.12	26.8
12	82	11.2	5.6	0.06	13.2
13	96	13.9	4.4	0.15	18.7
14	38	6.2	4.6	**1.00**	7.1
15	147	12.3	5.0	0.20	15.2
16	76	9.8	4.9	0.20	12.7
17	97	12.1	3.8	0.30	18.7
18	81	10.1	4.0	0.10	17.9
19	106	13.1	4.7	0.07	17.5
20	102	15.6	4.2	0.47	29.0
21	**227**	17.2	**3.6**	0.24	**31.0**
22	104	7.4	5.8	0.30	24.4
23	107	14.5	4.1	0.26	29.2
24	104	10.5	4.2	0.15	23.0
25	59	4.3	**3.6**	0.03	10.5
26	124	13.3	5.1	0.10	16.7
27	116	**18.5**	4.3	0.30	14.4
28	46	9.4	4.2	0.04	10.1
29	70	9.9	4.2	0.13	21.0
30	**35**	**5.8**	4.5	**0.01**	**6.8**
CV (±%)	31	42	17	33	35

CV = mean coefficient of variation between replicate sites (n = 4), bold: minima and maxima

Table 7

Median, minimum and maximum of different soil and microbial ratios in soils from 30 Pakistani arable sites

	Median	Min	Max
Soil organic C-to-total N	9.0	3.9	15.6
Soil organic C-to-total P	6.7	1.5	12.6
Microbial biomass C-to-soil organic C (%)	2.2	0.4	5.0
Microbial biomass N-to-total N	2.1	0.3	7.5
Microbial biomass P-to-total P	0.69	0.33	1.0
Microbial biomass C-to-N	8.7	3.1	21.3
Microbial biomass C-to-P	20.5	3.1	76.7
Ergosterol-to-microbial biomass C (%)	0.15	0.012	3.7
qCO_2 (mg CO_2-C d^{-1} g^{-1} biomass C)	92	11	407

Table 8

Oblique solution primary pattern matrix of the principal component analysis for the different soil physical, chemical and biological properties in soils from 30 Pakistani arable sites (orthotran/varimax transformation; n = 120); bold: definite assignation to a certain factor

	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
Microbial biomass C	**0.88**	-0.23	0.18	0.02	0.10
Microbial biomass N	**0.77**	-0.28	-0.08	0.14	-0.05
Total N	**0.75**	0.20	-0.11	-0.07	0.35
Soil organic C	**0.71**	0.00	0.21	-0.05	0.01
Salt-derived Na	0.02	**0.93**	-0.01	-0.04	0.00
Respiration	0.39	**-0.67**	0.00	-0.13	0.27
Total P	0.08	0.00	**0.94**	-0.02	0.03
Microbial biomass P	0.04	0.03	-0.02	**0.97**	0.03
Soil pH	0.00	0.16	-0.21	-0.13	**-0.77**
Ergosterol	0.30	-0.18	-0.33	-0.13	**0.68**
Eigenvalue	3.34	1.47	1.09	0.99	0.93

Table 9

Oblique solution primary pattern matrix of the principal component analysis for the ratios of the different microbial properties in soils from 30 Pakistani arable sites (orthotran/varimax transformation; n = 120); bold: definite assignation to a certain factor

	Factor 1	Factor 2	Factor 3
Microbial biomass C/soil organic C	**0.85**	0.17	0.00
Microbial biomass C/P	**0.74**	0.17	-0.41
qCO_2	**-0.71**	0.52	0.17
Ergosterol/microbial biomass C	**-0.71**	0.09	-0.22
Microbial biomass C/N	0.50	0.13	0.09
Salt-derived Na	-0.18	**-0.91**	0.06
Soil pH	0.09	-0.01	**0.94**
Eigenvalue	2.61	1.17	1.12

The microbial biomass C-to-P ratio exceeded the soil organic C-to-total P ratio nearly threefold, but the soil organic C-to-total N ratio and microbial biomass C-to-N ratio were both on a similar level around 9.0 (Table 7). For this reason, the microbial biomass C-to-soil organic C ratio and the microbial biomass N-to-total N ratio were also both on a similar level at approximately 2.2%. The microbial biomass P-total P ratio was three times smaller and showed the smallest soil-to-soil variations, contrasting the ergosterol-to-microbial biomass C ratio. The second principal component analysis revealed strong positive relationships between the ratios microbial biomass-to-soil organic C, microbial biomass C-to-P and to a certain extent also microbial biomass C-to-N as factor 1. This factor also carried negative loadings by the metabolic quotient qCO_2 and the ergosterol-to-microbial biomass C ratio (Table 1). Consequently, the interactions of carbon and nutrient availability with the microbial community structure

were characterised by factor 1. Salt-derived Na (but also any other of the various salinisation indices – results not shown) had some negative effects on the metabolic quotient qCO_2 summarised as factor 2, characterising again salinity effects on soil biological properties. With some negative effects on the microbial biomass C-to-P ratio soil, pH formed factor 3.

4. Discussion

The 30 Pakistani soils under arable land-use management revealed exceptionally low contents of soil organic matter in combination with strong alkaline and saline soil conditions. This may explain why the median content of microbial biomass C in these Pakistani soils reached less than 30% of the worldwide average (Wardle, 1998). Microbial biomass C concentrations between 300 and 400 µg g^{-1} are common in many subtropical soils from India (Srivastava and Singh, 1989; Singh and Singh, 1993; Srivastava and Lal, 1994; Goshal and Singh, 1995). However, low microbial biomass C contents are also not unusual, mean values between 100 and 160 µg g^{-1} soil being measured in alfisols from Nigeria (Wick et al., 1998) and in andosols from Costa Rica (using a k_{EC} value of 0.45; Mazzarino et al., 1993) and Nicaragua (Joergensen and Castillo, 2001). No direct relationships between texture, soil pH and salinisation and soil microbial biomass indices could be drawn in the present group of soils. This contrasts the observation of others who found positive effects of clay content (Van Veen et al., 1984; Müller and Höper, 2004) and negative effects of salinity (Batra and Manna, 1997; Rietz and Haynes, 2003; Sardinha et al., 2003) on the microbial biomass level. However, the absence of clay effects has been reported by others, especially if different climatic regions were compared (Insam et al., 1989; Wardle, 1998). Acidic conditions

are known to lower microbial biomass levels in forest soils (Anderson and Domsch, 1993; Anderson and Joergensen, 1997), while nothing is known about pH effects on soil microbial biomass indices under alkaline conditions.

The microbial biomass C-to-N ratio was on the same level as the soil organic C-to-total N ratio, i.e. relatively higher than in arable soils from humid temperate climates, where the microbial biomass C-to-N ratio is always lower than the soil organic C-to-N ratio (Smith and Paul, 1990; Joergensen and Mueller, 1996). Microbial biomass C-to-N ratios exceeding soil organic C-to-N ratios had been repeatedly observed in tropical forest soils (Salamanca et al., 2002; Dinesh et al., 2003; 2004). Low N availability in combination with high C availability led to increased microbial biomass C-to-N ratios in pure cultures of soil fungi and soil bacteria (Anderson and Domsch, 1980), but also in incubation experiments with complex soil microbial populations (Chander and Joergensen, 2001; Joergensen and Raubuch, 2002). However, it is currently completely unknown whether deficiencies in nutrients other than N or P affect the microbial biomass C-to-N ratio.

The microbial biomass C-to-P ratio of the present group of soils is within the range known for subtropical soils, especially in India (Singh and Singh, 1993; Srivastava and Lal, 1994; Goshal and Singh, 1995). The microbial biomass C-to-P ratio is generally higher in these subtropical arable soils than in humid temperate soils, values around 20 to 30 are common, indicating low P availability to the soil microbial community (Muhammad et al., 2005). In contrast to most observations in temperate humid climates (Joergensen et al., 1995), the microbial biomass C-to-P ratio exceeds the soil organic C-to-total P ratio markedly as in the volcanic ash soils of Nicaragua (Joergensen and Castillo, 2001). The microbial biomass C-to-P ratio is increased by low P availability, but also by low N availability in combination with high C availability (Anderson and

Domsch, 1980; Kapoor and Haider, 1982; Sparling and Williams, 1986; Srivastatava and Lal, 1994).

The median microbial biomass C-to-soil organic C ratio of the present Pakistani soils is similar to the average figures in Germany (Anderson and Domsch, 1989), indicating that the availability of soil organic matter to microorganisms is not directly restricted by the alkaline and saline conditions. However, the level of soil organic matter is three to four times lower indicating a very low C input by the crops. Another important feature of the Pakistani soils is the high level of the metabolic quotient qCO_2 in comparison to average figures of arable soils in Germany (Anderson and Domsch, 1990). Sublethal stress in the present group of alkaline and saline Pakistani soils lowers the efficiency of substrate use, i.e. more substrate must be catabolised to CO_2 and less substrate can be incorporated into the microbial biomass (Killham, 1985). This is the basic reason for the repeatedly observed negative relationship (Anderson and Domsch, 1990, 1993; Joergensen and Castillo, 2001; Chander et al., 2001) between qCO_2 and the microbial biomass C-to-soil organic C ratio as in the present group of alkaline and saline Pakistani soils. The positive relationship between qCO_2 and the ergosterol-to-biomass C ratio indicates that an increasing percentage of fungi in the total biomass may reduce the yield efficiency as described by Sakamoto and Oba (1994). The combination of qCO_2 values and high microbial biomass C-to-soil organic C ratio point to the threat of further reduction in the soil organic matter level, if the conditions for microbial activities are improved, for example by reducing salinity.

The median ergosterol-to-microbial biomass C ratio of the present Pakistani soils is only a quarter of the mean value obtained by Djajakirana et al. (1996) in German arable soils. Frey et al. (1999) found a decreasing ratio of fungal-to-bacterial biomass with increasing drought, and Sardinha et al. (2003) found a decreasing ratio of ergosterol-to-microbial biomass C with increasing salinity. This means that drought and salinity may

explain the generally very low ergosterol-to-biomass C ratios in our Pakistani soils. If ergosterol is recalculated into fungal biomass C by multiplication by 90 (Djajakirana et al., 1996), fungi represent 14% of total microbial biomass C in the present alkaline and saline Pakistani soils. However, the possibility cannot be excluded that the fungi of the Pakistani soils generally have very low ergosterol contents. The ergosterol content as an indicator of fungal biomass and changes in the microbial community structure was the only soil biological property affected differently by salinity and pH. In contrast to acidic soils, where salinisation overrides pH effects (Sardinha et al., 2003), increasing soil pH had stronger negative effects on ergosterol contents than salinisation under strongly alkaline conditions.

5. Conclusions

The level of soil organic matter and microbial biomass indices was generally very low in the saline and alkaline soils from 30 Pakistani arable sites, but no strong direct effects of salinity and alkalinity on microbial biomass indices could be observed. The microbial biomass C-to-soil organic C ratio was in the range of arable soils from temperate humid climate at neutral pH, indicating that saline and alkaline soil conditions did not reduce the accessibility of organic matter to soil microorganisms. Only basal respiration was negatively affected by salinity indices, but not by soil pH. High levels of the metabolic quotient qCO_2 indicate that the microorganisms are generally stressed by the saline and alkaline conditions, leading to low substrate use efficiencies. This intensifies the threat of further losses in soil fertility by the reduction in soil organic matter levels, which is emphasized by the classical negative relationship between qCO_2 and the microbial biomass C-to-soil organic C ratio. Also, on the basis of

the gross ratios microbial biomass C-to-N, microbial biomass C-to-P, and also ergosterol-to-microbial biomass C, the microbial community structure was not directly affected by salinity. Only an increasing pH under alkaline conditions has some negative effects on ergosterol contents in soil and thus on soil microfungi.

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Chapter 4

Decomposition of compost and plant residues in Pakistani soils along a gradient in salinity

Decomposition of compost and plant residues in Pakistani soils along a gradient in salinity

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Abstract

Three organic amendments (compost, maize straw and pea straw) were added to five Pakistani soils from a gradient in salinity to test the following two hypotheses: (1) Increasing salinity at high pH decreases proportionally the decomposition of the added organic amendments and the resulting net increase in microbial biomass; (2) salinity effects override differences in quality of the organic amendments. Although salinity has depressive effects on microbial biomass C, biomass N, biomass P, and ergosterol, the clear gradient according to the soil salt concentration was not reflected by the soil microbial properties. Nevertheless, the ratios microbial biomass C-to-N and biomass C-to-P decreased continuously with increasing salinity. In contrast, the ergosterol-to-microbial biomass C ratio was constant in the four soils at pH > 8.9, but nearly doubled in the most saline, but least alkaline soil. The addition of the three organic amendments always increased the contents of the microbial indices analysed. The amendment-induced increase was especially strong for microbial biomass P and reflected the total P content of the added substrates. Highest sampling date-specific mean contents of microbial biomass C and biomass N were measured at day 0, immediately after the amendments were added. The organic amendments increased the CO_2 evolution rates of all 5 soils in a soil-specific and amendment-specific way, but without a clear effect of salinity. The same was true for total C and total N in the two fractions of particulate organic matter (POM) 63 – 400 µm and > 400 µm. Lowest percentage of substrate derived CO_2-C-and highest recovery of POM-C was observed in the compost treatment and the reverse in the pea straw treatment. The percentage of N recovered as POM was much smaller than the percentage of C in the compost and in the pea straw treatment, but not in the maize straw treatment.

Key words: Sodicity • Microbial biomass • Ergosterol • CO_2 evolution • POM

Introduction

In arid and semi-arid Pakistan, salinisation affects 30% of arable land and is a major threat to plant growth (Sandhu and Qureshi 1986; Qureshi and Barret-Lenard 1998). The negative effects of salinisation are intensified by the low levels of soil organic matter (Muhammad et al. 2005) and increasing lability of soil structure, i.e. the tendency to slake, disperse and swell under specific conditions (Keren 2000; Qadir and Schubert 2002). Unfortunately, the practice of adding organic amendments, such as compost or plant residues, for improving soil organic matter levels and thus maintaining soil fertility is rarely adopted in Pakistan.

In contrast to soil physical and chemical properties, soil microbiological aspects of saline environments have been less intensively studied (Zahran 1997), but recent studies clearly revealed the adverse effects of salinisation on the soil microbial biomass (Sarig et al. 1996; Batra and Manna 1997; Rietz and Haynes 2003). In particular, the fungal part of the microbial biomass estimated by PLFA (Badran 1994; Pankhurst et al. 2001) or ergosterol analysis (Sardinha et al. 2003) was strongly reduced in saline soils. Sardinha et al. (2003) concluded that salinisation is one of the most stressing environmental conditions for soil microorganisms. In contrast to the effects on microbial and especially fungal biomass, the effects of salinisation on the decomposition of added plant material are contradictory, i.e. both increases (Nelson et al. 1996) and decreases (Pathak and Rao 1998) have been reported. These contradictory observations might be due to differences in quality of the added organic amendments or differences in the soil properties, especially the levels of salinity and soil pH.

Under the arid or semiarid climate of Pakistan, salinity is usually combined with high pH conditions, due to the presence and enrichment of calcium carbonate in the uppermost soil layers (saline soils) or to hydrolysis of sodium carbonate (sodic soils). Three organic amendments (compost, maize straw and pea straw) differing in quality of the organic components and nutrient content, especially N and P, were added to five alkaline Pakistani soils forming a gradient in salinity to test the following two hypotheses: (1) Increasing salinity at high pH decreases proportionally the decomposition of the added organic amendments and consequently the net increase in microbial biomass; (2) salinity effects override differences in substrate quality of the organic amendments.

Material and methods

Substrates and analyses

Soils included in the present study were collected from central Pakistan, in the region of Gujranwala, Punjab, forming a gradient in salinity (Table 1). Soil properties (Table 1) are determined as described by Muhammad et al. (2005). The local climate is characterised by two distinct seasons, a very hot summer from June to August with maximum temperatures up to 46°C and a cool period from October to February with minimum temperatures as low as 5°C. The mean annual precipitation of 720 mm is unevenly distributed over the year, i.e. approximately 50% comes in July. In July 2002, the 5 soils were sampled at 0-15 cm depth with a soil corer (4 x 15 cm), sieved (< 2 mm) and transported to Witzenhausen, Germany.

Three different organic amendments were used for the subsequent incubation experiment. Biogenic municipal waste compost was taken from the compost plant in the vicinity of Witzenhausen (Gattinger et al. 2004). Maize leaf straw (*Zea mays* L.) and pea straw (*Pisum sativum* L.) were obtained from the experimental farm of the faculty. The organic amendments were air-dried and ground < 5 mm. Aliquots of the three organic amendments were dried at 60°C, ground in a Wiley mill to pass a 0.5-mm screen before elemental analysis (Table 2). Total C and total N were determined using a Vario Max CN analyser (Elementar, Hanau, Germany). Concentrations of total P, total S, K, Mg, and Ca were determined after HNO_3/pressure digestion according to Heinrichs et al. (1986) as described by Chander et al. (2001) and analysed by ICP-AES (Spectro Analytical Instruments/Kleve).

Incubation procedure

A laboratory incubation experiment with 5 replications was conducted with the following four treatments: (1) non-amended control, (2) + 1% compost, (3) + 1% maize leaf straw, (4) + 1% pea straw. For each treatment, 600 g (on an oven-dry basis) of soil at 50% water holding capacity plus the different amendments were weighed in 3 l incubation vessels and incubated for 56 days at 30°C in the dark. The CO_2 evolved was absorbed in NaOH solution and back-titrated with HCl. The NaOH was changed after the 2^{nd}, 5^{th}, 8^{th}, and 14^{th} day and thereafter weekly. Soil samples of 100 g fresh-weight were taken after 0, 5, 14, 28, and 56 days for analyses.

Microbial biomass determination

For estimating microbial biomass C (Vance et al. 1987) and biomass N (Brookes et al. 1985) by fumigation-extraction, two portions equivalent to 25 g oven dry soil were taken from the 100-g soil sample of the incubation. One portion was fumigated for 24 h at 25°C with ethanol-free $CHCl_3$. The fumigant was removed before 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 through a folded filter paper (Schleicher & Schuell 595 ½, Dassel, Germany). The non-fumigated portion was extracted similarly at the time fumigation commenced. Organic C in the extracts was measured as CO_2 by infra-red absorption after combustion at 850°C using a Dimatoc 100 automatic analyzer (Dimatec, Essen, Germany). Microbial biomass C was E_C / k_{EC}, where E_C = (organic C extracted from fumigated soil) – (organic C extracted from non-fumigated soil) and k_{EC} = 0.45 (Wu et al., 1990, Joergensen 1996). Total N in the extracts was measured as activated NO_2^* by chemoluminescence detection (Dima-N, Dimatec) after combustion at 850°C. Microbial biomass N was E_N / k_{EN}, where E_N = (total N extracted from fumigated soil) - (total N extracted from non-fumigated soil) and k_{EN} = 0.54 (Brookes et al. 1985; Joergensen and Mueller 1996).

For estimating microbial biomass P (Brookes et al. 1982) by fumigation-extraction, three portions equivalent to 2.5 g oven dry soil were taken from the 100-g soil sample of the incubation. The first 2.5-g portion was fumigated as described above, extracted with 50 ml 0.5 M $NaHCO_3$ (pH 8.5) by 30 min horizontal shaking at 200 rev min^{-1}, centrifuged for 15 min at (2000 *g*), and filtered (Schleicher & Schuell 595 ½). One non-fumigated portion was extracted similarly at the time fumigation commenced. The remaining portion was extracted after addition of 25 µg P g^{-1} (0.5 ml KH_2PO_4) in the

same way as non-fumigated samples. Phosphate was measured by photo-spectrometry at 882 nm as described by Joergensen et al. (1995).

Ergosterol was measured in 2 g moist soil taken from the 100-g soil sample of the incubation. Ergosterol was extracted with 100 ml ethanol for 30 minutes by horizontal shaking at 250 rev min^{-1} and filtered (Whatman GF/A) (Djajakirana et al., 1996). Quantitative determination was performed by reversed-phase HPLC analysis at 26°C using a column of 125 mm x 4 mm Sphereclone 5µ ODS II with a guard column of 4 mm x 3mm. The chromatography was performed 100% with methanol and a resolution of detection of 282 nm.

Particulate organic matter

Soil (100 g) was initially dispersed in 100 ml 5% NaCl, shaken by hand and allowed to stand for 45 min or over night. Then the samples were poured gradually onto sieves of 400 µm and 63 µm mesh sizes (Magid and Kjaergaard, 2001; Magid et al., 2004) and washed with tap water. The aggregates were destroyed by pushing the soil through the sieve during the washing procedure until the water passing through the sieve became clear. The material retained on the sieve was transferred into a beaker. Tap water was added, the bucket was swirled and organic material was separated from the mineral material by flotation-decantation. Swirling and flotation-decantation was repeated several times, until organic particles were no longer visible in the mineral fraction. Then, the mineral fraction was discarded. The remaining two fractions: particulate organic matter (POM) 63 – 400 µm and POM > 400µm were transferred into a crucible, dried at 60°C, and ground for further analysis (total C and total N).

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 experimental effects on microbial biomass indices was tested by a two-way ANOVA with soils and organic amendment treatments as independent main factors and sampling day as repeated measures, followed by a main effect-specific one-way analysis of variance using the Tukey/Kramer HSD-test (honestly significant difference). The significance of experimental effects on CO_2 evolution rate and particulate organic matter recovery were analysed after log-transformation of the data by a soil-specific one-way ANOVA using the Tukey/Kramer HSD-test. All statistical analyses were performed using StatView 5.0 (SAS Inst. Inc.).

Results

Microbial biomass indices and extractable fractions

Although salinity has depressive effects on microbial biomass C, biomass N, biomass P, and ergosterol, the clear gradient according to the soil salt concentration was not reflected by the soil microbial properties (Table 1). The soil-specific mean contents of microbial biomass C and biomass N formed two groups; one with higher microbial biomass contents at low salt concentrations and the other with lower microbial biomass contents at high salt concentrations (Table 3). The microbial biomass C-to-N ratio decreased continuously with increasing salt content (Table 4). The soil-specific mean

contents of microbial biomass P and ergosterol did not differ significantly between the 5 soils (Table 3). However, the microbial biomass C-to-P ratio declined similarly to the microbial biomass C-to-N ratio with increasing salinity (Table 4). In contrast, the ergosterol-to-microbial biomass C ratio was constant in the soils at pH > 8.9, but nearly doubled in the most saline, but least alkaline soil 16. The soil-specific mean contents of 0.5 M K_2SO_4 extractable C did not differ significantly between the 5 soils (Table 3). This was not true for the contents of 0.5 M $NaHCO_3$ extractable P, which showed a significant difference between the two soils with lowest and highest salinity.

The addition of the three organic amendments always increased the contents of the microbial indices analysed, but the compost-induced increase of the amendment-specific mean was not significant for microbial biomass C and ergosterol (Table 3). The strongest increase in ergosterol was observed after the addition of maize straw, also leading to a maximum ergosterol-to-microbial biomass C ratio (Table 4). In contrast, pea straw had a depressive effect on the ergosterol-to-microbial biomass C ratio, although pea straw caused the strongest increase in all the other microbial indices (Table 3). The amendment-induced increase was especially strong for the contents of 0.5 M K_2SO_4 extractable C and microbial biomass P. The absolute amendment-specific increase in microbial biomass P was always roughly twice that of 0.5 M $NaHCO_3$ extractable P. The amendment-specific mean microbial biomass C-to-P ratio varied highly in a 3-fold range and was lowest in the compost treatment (Table 4), followed by the pea straw and the maize straw treatments, reflecting the total P content of the added substrate (Table 2). In contrast, the microbial biomass C-to-N ratio varied in a much smaller range and did not reflect the total N content of the added substrate, i.e. this ratio was as high in the pea straw treatments as in the control treatment (Table 4).

Highest sampling-date specific mean contents of 0.5 M K_2SO_4 extractable C were measured immediately after addition of the three amendments, especially in the pea

straw treatment, but also in the maize straw treatment (Fig. 1). In these two treatments, the content of extractable C declined by 83 and 68%, respectively, within 5 days. Also, highest sampling date-specific mean contents of microbial biomass C and biomass N were measured at 0, immediately after the amendments were added (Table 3). This was mainly due to the large microbial biomass content of the pea straw (Fig. 2). In the maize straw treatments, maximum microbial biomass C contents were measured at day 5. In the compost treatments, maximum values were measured soil-specifically at day 0 or day 5. From day 14 on, the microbial biomass C and biomass N contents remained more or less constant in all three amendment treatments (Fig. 2, Table 1). The sampling date-specific mean microbial biomass C-to-N ratio varied between 7.7 and 8.6, without a clear trend throughout the incubation period. The sampling date-specific mean content of microbial biomass P increased until day 27.4 and declined again in all 4 amendment treatments (Table 4), this being most pronounced in the compost treatment (Fig. 3). In the pea straw treatment, the microbial biomass C-to-P ratio was highest at day 5, in the other two amendment treatments maximum microbial biomass C-to-P ratios were reached soil-specifically between day 0 and 14 (Fig. 4).

Particulate organic matter and CO_2 evolution

The addition of organic amendments increased the CO_2 evolution rates of all 5 soils in a soil-specific and amendment-specific way, without clear effects of salinity, although highest CO_2 evolution rates were measured in the two soils with lowest salt content and lowest CO_2 evolution rates were always found in soil 5 with highest salt content (Table 5). The same was true for total C and total N in the two POM fractions. However, the absolute and relative increase was much stronger in the fraction > 400 µm than in the

fraction 63 – 400 µm. These two fractions contributed on average 1.1% and 10.2%, respectively, to soil organic C of the control soils and 0.6%, but only 0.7%, respectively, to total N. Assuming that the addition of the organic amendments did not affect the decomposition of soil organic matter, the C recovered as CO_2, POM and microbial biomass C in the different amendment-treatments can be calculated. Lowest percentage of substrate derived CO_2-C-and microbial biomass C and highest recovery of POM-C was observed in the compost treatment and the reverse in the pea straw treatment (Fig. 5). However, the difference to the maize straw treatment was small for all four fractions. The percentage of N recovered as POM was much smaller than the percentage of C in the compost and in the pea straw treatment, but not in the maize straw treatment (Fig. 6). The net increase in microbial biomass C due to the organic amendments varied between 1.5% and 5.9% of the C added (Fig. 5). In contrast, the net increase in microbial biomass N was considerably higher and ranged from 20% to 185% of the N added (Fig. 6).

Table 1. Properties of 5 saline and alkaline soils from Pakistan used in the incubation experiment

Properties	Soil 1	Soil 2	Soil 3	Soil 4	Soil 5
Sand (%)	44	29	30	26	25
Silt (%)	38	57	54	62	65
Clay (%)	18	14	16	12	10
$CaCO_3$ (%)	12.5	8.5	7.6	4.4	16.1
pH-H_2O	9.0	9.1	9.2	9.3	8.2
Electrical conductivity (mS cm^{-1})	5.7	5.7	8.6	13.1	16.0
SAR	2.7	4.5	6.7	11.0	8.5
Salt (mg g^{-1} soil)	2.1	2.8	3.1	4.0	6.0
Salt-Na (mg g^{-1} soil)	0.31	0.48	0.64	0.98	1.45
Salt-HCO_3 (mg g^{-1} soil)	0.13	0.15	0.12	0.13	0.10
Soil organic C (mg g^{-1} soil)	6.8	6.6	4.6	3.7	5.4
Total N (mg g^{-1} soil)	0.71	0.57	0.43	0.39	0.55
Total P (mg g^{-1} soil)	0.71	0.81	0.78	0.75	0.67

SAR = sodium absorption ratio

Table 2. Chemical properties of the organic amendments used in the incubation experiment.

Properties	Compost	Maize straw	Pea straw
Total C (%)	16.6	45.0	44.0
Total N (mg g^{-1} dry weight)	16	11	39
Total P (mg g^{-1} dry weight)	6.0	0.75	4.5
Total S (mg g^{-1} dry weight)	3.9	0.97	2.3
K (mg g^{-1} dry weight)	15.7	17.2	29.4
Mg (mg g^{-1} dry weight)	8.8	1.8	2.1
Ca (mg g^{-1} dry weight)	46.1	6.1	8.7
Total C/N	10.3	41.5	11.3
Total C/P	27	600	98
Total C/S	43	464	191

Table 3. Means for main effects of soils and organic amendment treatments as main factors, sampling days as repeated measures on the contents of microbial biomass C, 0.5 M K_2SO_4 extractable C, microbial biomass N, biomass P, 0.5 M $NaHCO_3$ extractable P (P-Olson), and ergosterol; ND = not determined; CV = mean coefficient of variation between replicate measurements (n = 4), different letters within a column indicate a main effect-specific significant difference ($P < 0.05$; Tukey/Kramer HSD-test); degrees of freedom: soils (4), organic amendments (2), sampling days (4, ergosterol 1)

Main effects	Microbial biomass C (µg g^{-1} soil)	0.5 M K_2SO_4 extractable C (µg g^{-1} soil)	Microbial biomass N (µg g^{-1} soil)	Microbial biomass P (µg g^{-1} soil)	0.5 M $NaHCO_3$ extractable P (µg g^{-1} soil)	Ergosterol (µg g^{-1} soil)
Soils						
Soil 1	374 b	136 a	45 b	22.8 a	15.5 b	0.42 a
Soil 2	367 b	118 a	45 b	22.5 a	14.6 b	0.48 a
Soil 3	256 a	122 a	33 a	22.8 a	13.2 ab	0.34 a
Soil 4	259 a	127 a	36 a	21.1 a	13.7 ab	0.32 a
Soil 5	216 a	135 a	33 a	21.9 a	10.8 a	0.46 a
Organic amendments						
Control	139 a	42 a	16 a	7.5 a	7.0 a	0.22 a
Compost (1%)	176 a	67 b	23 b	23.4 c	14.1 b	0.24 a
Maize straw (1%)	379 b	132 c	53 c	15.0 b	8.4 a	0.76 c
Pea straw (1%)	483 c	270 d	61 d	43.0 d	24.8 c	0.39 b
Sampling days						
Day 0	393	352	50	18.9	12.9	0.28
Day 5	347	94	44	20.7	14.8	ND
Day 14	225	77	33	21.7	13.7	ND
Day 28	266	61	33	27.4	14.2	ND
Day 56	241	55	32	22.4	12.1	0.51
Analysis of Variance						
Soils	<0.001	0.107	<0.001	0.143	<0.001	<0.001
Organic amendments	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Sampling **d**ays	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
S x **O**	0.001	0.929	0.019	0.295	0.987	0.107
S x **SD**	<0.001	0.293	<0.001	0.982	0.993	<0.001
O x **SD**	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
CV (±%)	16	20	23	18	22	30

Table 4. Means for main effects of soils and organic amendment treatments as main factors, sampling days as repeated measures on the ratios microbial biomass C/N, biomass C/P, and ergosterol/microbial biomass C; ND = not determined; CV = mean coefficient of variation between replicate measurements (n = 4), different letters within a column indicate a main effect-specific significant difference ($P < 0.05$; Tukey/Kramer HSD-test); degrees of freedom: soils (4), organic amendments (2), sampling days (4, ergosterol 1)

	Microbial biomass C/N	Microbial biomass C/P	Ergosterol/microbial biomass C (%)
Soils			
Soil 1	9.4 b	16.3 b	0.13 a
Soil 2	8.9 b	15.9 b	0.15 a
Soil 3	8.5 b	11.1 a	0.14 a
Soil 4	7.4 a	12.2 a	0.14 a
Soil 5	6.5 a	9.7 a	0.26 b
Organic amendments			
Control	8.9 b	17.4 c	0.17 bc
Compost (1%)	7.8 a	7.6 a	0.14 b
Maize straw (1%)	7.4 a	25.0 d	0.23 c
Pea straw (1%)	8.5 b	11.2 b	0.10 a
Sampling days			
Day 0	8.3	20.6	0.07
Day 5	8.6	16.8	ND
Day 14	7.7	10.4	ND
Day 28	8.4	8.6	ND
Day 56	7.7	10.5	0.21
Analysis of Variance			
Soils	<0.001	<0.001	<0.001
Organic amendments	<0.001	<0.001	<0.001
Sampling **d**ays	0.002	<0.001	<0.001
S x **O**	<0.001	<0.001	0.207
S x **SD**	<0.001	<0.001	0.067
O x **SD**	<0.001	<0.001	<0.001
CV (± %)	11	9	20

Table 5. CO_2-C evolution during 56 days of incubation experiment, total C and total N in the two fractions of particulate organic matter (POM) 63 – 400 µm and > 400 µm; CV = mean coefficient of variation between replicate measurements (n = 4); different letters within a column indicate a soil-specific significant difference ($P < 0.05$; Tukey/Kramer HSD-test); degrees of freedom: soils (4), organic amendments (3).

	Total N in POM		Total C in POM		
	63 – 400 µm ($\mu g\ g^{-1}$ soil)	> 400 µm ($\mu g\ g^{-1}$ soil)	63 – 400 µm ($\mu g\ g^{-1}$ soil)	> 400 µm ($\mu g\ g^{-1}$ soil)	ΣCO_2-C ($\mu g\ g^{-1}$ soil)
Soil 1					
Control	4.4 a	4.9 a	590 a	123 a	303 a
Compost (1%)	7.7 b	94.1 c	961 ab	1384 c	461 b
Maize straw (1%)	7.7 b	33.2 b	1037 b	692 b	2552 c
Pea straw (1%)	7.1 ab	37.5 b	851 ab	606 b	2758 c
Soil 2					
Control	6.1 a	6.5 a	869 a	118 a	323 a
Compost (1%)	9.2 b	110.6 c	1085 a	1478 c	497 b
Maize straw (1%)	6.6 ab	51.6 b	928 a	1188 c	2490 c
Pea straw (1%)	7.3 ab	31.2 b	898 a	513 b	2664 c
Soil 3					
Control	2.6 a	1.4 a	536 a	28 a	279 a
Compost (1%)	5.5 b	31.1 b	697 a	759 b	393 b
Maize straw (1%)	3.4 a	32.7 b	463 a	657 b	2450 c
Pea straw (1%)	4.8 b	27.0 b	570 a	487 b	2709 c
Soil 4					
Control	2.6 a	0.9 a	354 a	17 a	246 a
Compost (1%)	6.1 b	103.2 c	677 b	1376 c	406 b
Maize straw (1%)	4.5 ab	51.8 bc	551 ab	1322 c	2357 c
Pea straw (1%)	4.2 ab	36.4 b	445 ab	663 b	2681 c
Soil 5					
Control	2.9 a	2.2 a	434 a	41 a	222 a
Compost (1%)	6.8 b	98.9 c	889 b	1530 c	332 b
Maize straw (1%)	3.1 a	37.1 b	619 ab	932 bc	2027 c
Pea straw (1%)	3.9 a	38.3 b	422 a	622 b	2469 c
Analysis of Variance					
Soils	<0.001	<0.001	<0.001	<0.001	<0.001
Organic amend.	<0.001	<0.001	<0.001	<0.001	<0.001
S x **O**	<0.418	<0.001	0.355	0.001	<0.001
CV (± %)	16	15	14	10	10

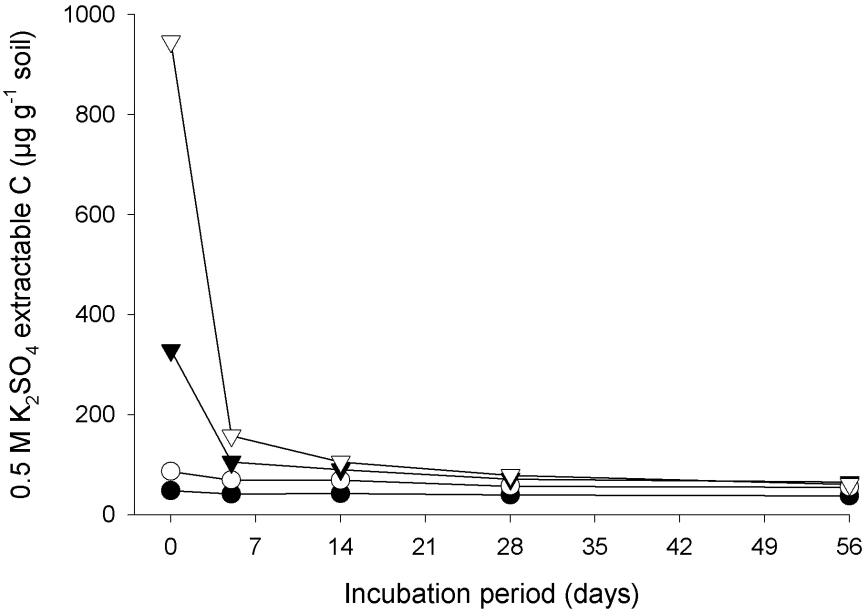

Fig. 1. Organic amendment-specific time-course in the content of 0.5 M K_2SO_4 extractable C during a 56-day incubation at 30°C, mean of all 5 soils for the control (●), compost (○), maize straw (▼), and pea straw (▽) treatment

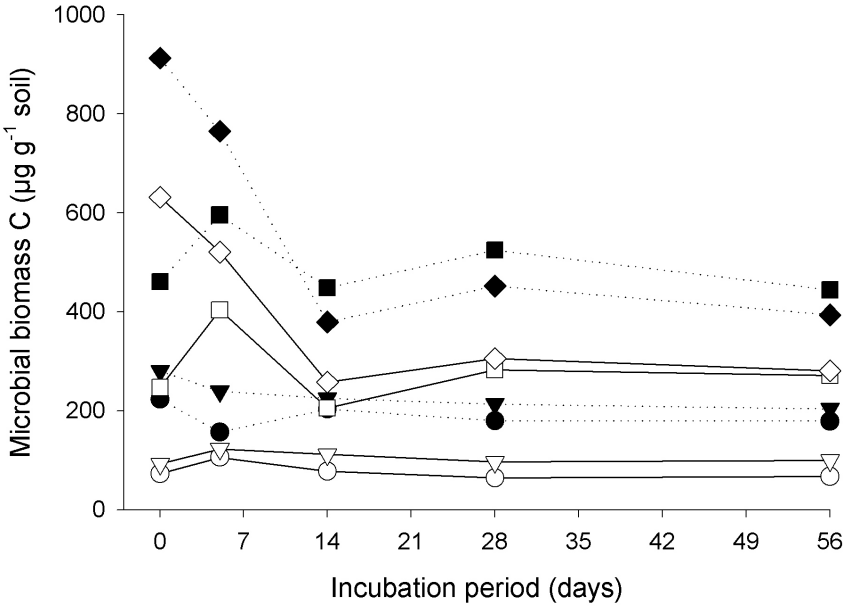

Fig. 2. Soil and organic amendment-specific time-course in the content of microbial biomass C during a 56-day incubation at 30°C, soil 1-control (●), soil 1-compost (▼), soil 1-maize straw (■), soil 1-pea straw (◆), soil 5-control (○), soil 5-compost (▽), soil 5-maize straw (□), soil 5-pea straw (◇))

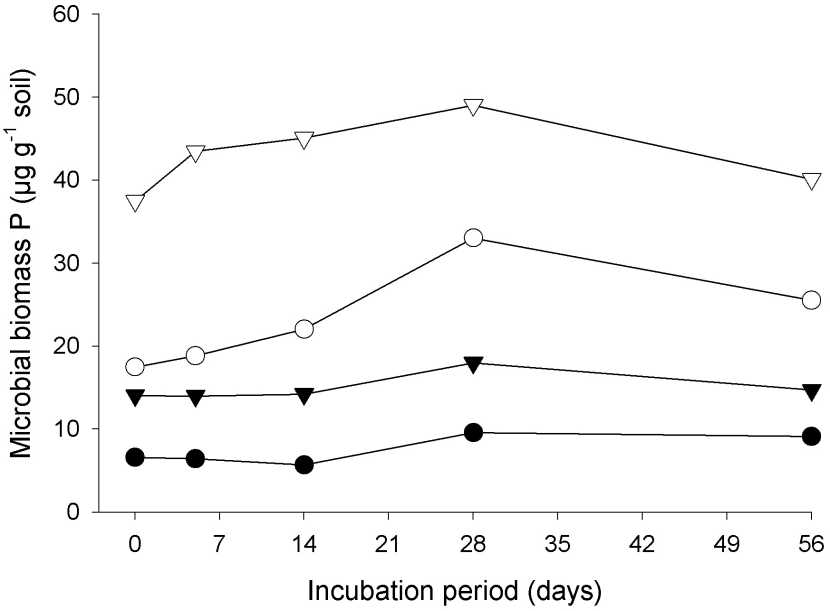

Fig. 3. Organic amendment-specific time-course in the content of microbial biomass P during a 56-day incubation at 30°C, mean of all 5 soils for the control (●), compost (○), maize straw (▼), and pea straw (▽) treatment

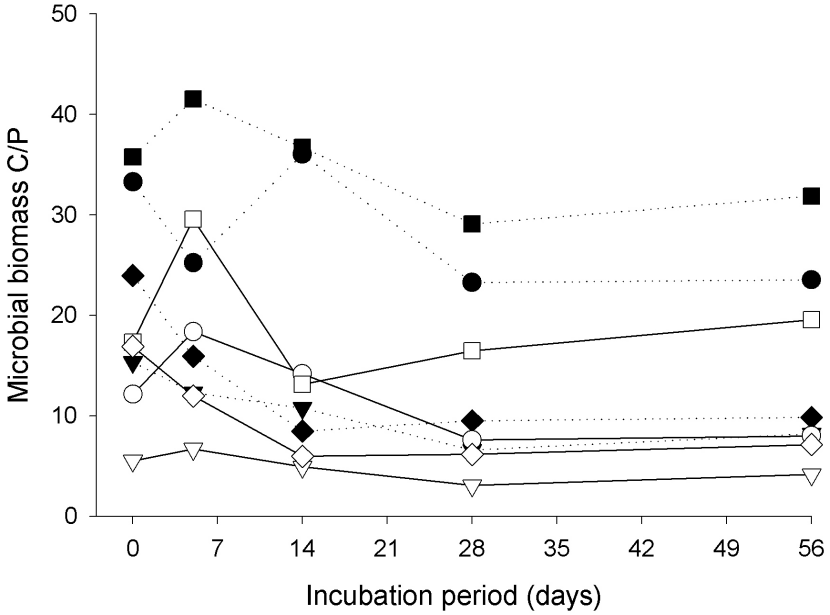

Fig. 4. Soil and organic amendment-specific time-course in the microbial biomass C/P ratio during a 56-day incubation at 30°C, soil 1-control (●), soil 1-compost (▼), soil 1-maize straw (■), soil 1-pea straw (◆), soil 5-control (○), soil 5-compost (▽), soil 5-maize straw (□), soil 5-pea straw (◇)

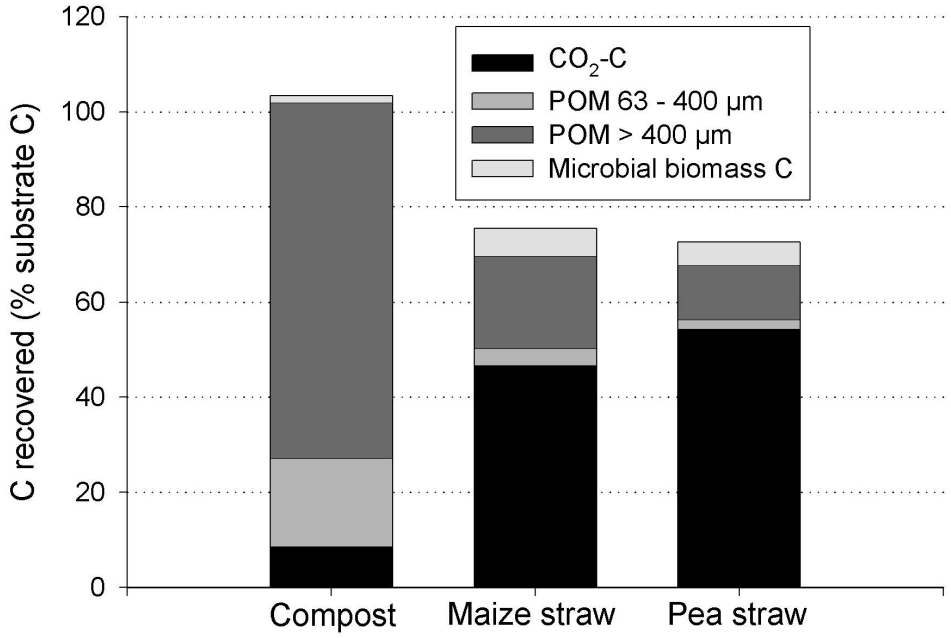

Fig. 5. C recovered at the end of the 56-day incubation at 30°C as CO_2-C and as particulate organic matter (POM) in the fractions 63 – 400 µm and > 400 µm

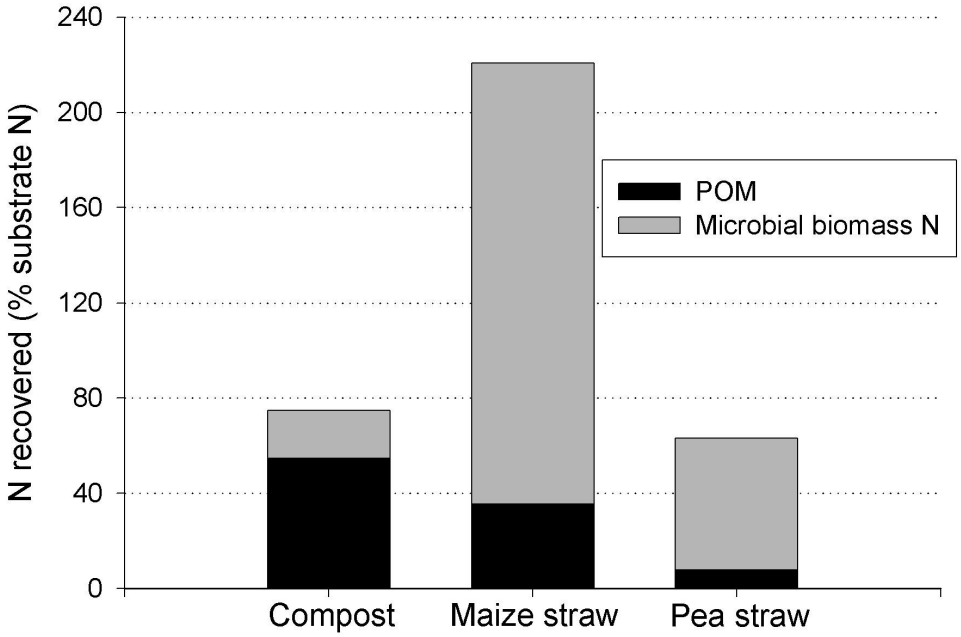

Fig. 6. Organic N recovered at the end of the 56-day incubation at 30°C as particulate organic matter (POM) > 63 µm

Discussion

The salinity effects on mineralization of maize straw or pea straw to CO_2 were relatively stronger than those on compost, but the salinity effects were generally not very strong and considerably smaller than those on microbial biomass indices. CO_2C mineralization is often not a very sensitive indicator of environmental stress for soil microorganisms, due to the minute reaction of this process on e.g. pesticide application (Harden et al. 1993), increasing acidity (Wolters 1991) or increasing heavy metal contamination (Chander et al. 2002a).

The net increase in microbial biomass C and biomass N was much more strongly affected than C mineralization and showed a significant retardation above a certain threshold of salinity, i.e. above an electric conductivity of approximately 4 mS cm^{-1}. However, not only the amount of salt in soil seems to be important but also the composition of the salt, especially its sodium concentration. However, the differences in net increase may be caused to a large percentage by the existing initial differences in soil microbial properties, i.e. the much lower microbial biomass levels of the more saline soils 14 to 15. It has been shown repeatedly that the net increase in microbial biomass depends on the ratio of substrate to initial microbial biomass, i.e. the lower the biomass the smaller the net increase (Witter and Kanal 1998; Chander and Joergensen 2001). The present results do not indicate strong direct salinity effects on soil microorganisms. The lower levels of microbial biomass and soil organic matter in the more saline soils are presumably mainly caused indirectly by a reduced crop growth and as consequently a reduced C input in the soil.

One reason for the small effects of salinity on decomposition processes might be the strong microbial colonisation of added substrates as indicated by maximum values of microbial biomass at day 0 immediately after incorporation of the organic

amendments into the soil. Annual crop plants can be strongly colonised by epiphytic organisms, especially during the period of maturity, which seem to play an important role for decomposition in soil (Flessa et al. 2002). If living green plant material were to be added, it cannot be completely ruled out that a part of the $CHCl_3$-labile material would originate from still intact and living plant material (Appuhn and Joergensen 2005). Wu et al. (1993) stated that the addition of ryegrass led to a newly synthesised microbial biomass merely added to that already present. The present results clearly indicate that the microbial biomass added by a complex naturally grown organic amendment is not necessarily newly synthesised. This is in accordance with the results of Potthoff et al. (2001), who measured a 30% increase in microbial biomass C one day after the addition of ^{14}C-labelled wheat straw.

The amount of P added to the soil strongly affects the content of microbial biomass P and the microbial biomass C-to-P ratio, which is generally in the range known from arid subtropical countries, especially India (Singh and Singh 1993; Srivastava and Lal 1994). However, in contrast to most observations in temperate humid climates, the microbial biomass C-to-P ratio exceeds the soil organic C-to-total P ratio, as in the volcanic ash soils of Nicaragua (Joergensen and Castillo 2001). The two P-rich organic amendments compost and pea straw decreased the microbial biomass C-to-P ratio, the relatively P-poor maize straw increased this ratio. It has been reported that the microbial biomass C-to-P ratio is increased by low P availability, but also by low N availability in combination with high C availability (Anderson and Domsch 1980; Kapoor and Haider 1982; Sparling and Williams 1986; Srivastatava and Lal 1994). The changes in microbial biomass C-to-P ratio during the incubation indicate some changes in the microbial community structure, as observed by others in non-saline soils on the basis of other indices such as the biomass C-to-N ratio, the PLFA composition or the ergosterol-

to-microbial biomass C ratio (Rost et al. 2001; Chander et al. 2002b; Waldrop and Firestone 2004).

Ergosterol is an important component of fungal cell membranes responsible for their stability (Weete and Weber 1980). It has been determined as a specific index for fungal biomass in a variety of solid substrates, including soils (Seitz et al. 1977; Grant and West 1986). The ergosterol-to-microbial biomass C ratio ranges from 0.1% in wetland soils (Chander et al. 2001) 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 11% of total soil microbial biomass C at the beginning and 18% at the end of the incubation experiment. This low level of ergosterol-to-microbial biomass C ratios is typical for tropical soils (Joergensen and Castillo 2001; Salamanca et al. 2002) and also for saline soils (Sardinha et al. 2003). The data of Sardinha et al. (2003) clearly revealed that salinity overrides pH effects at acidic levels of pH 5, in contrast to the present data, indicating that strongly alkaline conditions around and above pH 9 have stronger effects on the ergosterol-to-microbial biomass C ratio than salinity. The addition of maize residues is known to promote the growth of soil fungi, especially ergosterol-containing fungi (Karlen et al. 1994), which is reflected by the results of the present experiment. The depressive effects of pea straw on the ergosterol level are less well documented in the literature. Schutter and Dick (2001) found increased contents of the fungal PLFA markers 18:1ω9c and 18:2ω6c after pea straw addition and no significant difference to their triticale treatment.

The mineralization rates of the present compost were in the range of C-specific CO_2 production rates at different maturity stages of biogenic municipal waste composts (Gattinger et al. 2004) and also similar to that observed by Rasul et al. (2005) who measured a 20% loss of CO_2-C in a treatment with biogenic municipal waste compost

from the same compost plant at a 1% addition rate during a 63-day incubation at 25°C. Also the much higher mineralization rates of maize straw and pea straw to CO_2 are within the range reported in the literature considering the differences in incubation conditions (Vanlauwe et al. 1994; Goh and Tutua 2004; Potthoff et al. 2005). The total decomposition of pea straw determined by the recovery of particulate organic matter was 86.6% and thus similar to that of green alfalfa leaves (Muhammad et al. 2005b).

If we assume that the balance gap between CO_2 and the amount of particulate organic matter can be fully assigned to microbial products, i.e. a small fraction as biomass and the majority as microbial residues, the yield coefficient can be calculated as follows (van Veen et al. 1984; Joergensen et al. 1990):

Y = yield coefficient: substrate C in microbial products / substrate C = A / B

A = 100 – (% as CO_2-C) – (% as C in particulate organic matter)

B = 100 – (% as C in particulate organic matter)

According to this calculation, the yield coefficient for maize straw was Y = 0.39 (A / B = 30.4% / 77.1%) and that for pea straw Y = 0.37 (A / B = 32.3% / 86.6%). This yield coefficient is close to the estimated value proposed by Paul and Clark (1996) of 0.4, but much closer than the yield coefficient of 0.55 for the soil microbial biomass after a 10-day incubation (Jenkinson and Powlson 1976) or the yield coefficient of 0.70 for glucose after a 2-day incubation (Chander and Joergensen 2001). No yield coefficient could be calculated for the compost because no balance gap occurred between CO_2 evolution rate and POM. One possibility could be that microbial residues were directly attached to the compost particles after their formation. The quality of the recovered compost particulate organic matter was much different to the original compost, only

55% of added N being found in this fraction in contrast to more than 90% C, indicating strong preferential transformation of N components by soil microorganisms.

Conclusions

Salinity under alkaline conditions has strong effects on the incorporation of organic amendments into the microbial biomass, but only slight effects on the mineralization of compost, maize straw or pea straw to CO_2. Both processes are not linearly related to increasing salinity. Salt effects on soil biological properties are significant only above a threshold of 10 mS cm^{-1} electrical conductivity or a salt content of 3 mg g^{-1} soil. The salinity effects on mineralization of maize straw or pea straw to CO_2 were stronger than those on compost. The combined observation of the decomposition process by CO_2 evolution rate and the recovery of unused substrate as POM are useful and make it possible to calculate yield coefficients for complex organic substrates.

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Chapter 5

Interactions of compost and triple superphosphate on the growth of maize (*Zea mays*) in a saline Pakistani soil

Interactions of compost and triple superphosphate on the growth of maize (*Zea mays*) in a saline Pakistani soil

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Abstract

A greenhouse pot experiment was designed with different combinations of compost and triple superphosphate amendments to investigate the interactions between plant growth, microbial biomass formation and compost decomposition in a strongly saline Pakistani arable soil in comparison to a non-saline German arable soil. The Pakistani soil had a two times lower content of ergosterol, four times lower contents of microbial biomass C, biomass N and biomass P, but nearly a 20 times lower content of $NaHCO_3$ extractable P. The addition of 1% compost always had positive effects on the microbial properties and also on the content of $NaHCO_3$ extractable P. The addition of superphosphate solely or in the treatments +compost +P or +P-enriched compost induced a strong and similar absolute increase in microbial biomass P in both soils. The yield in the control treatment of the German soil was more than 10 times larger than in the Pakistani soil. The amendment treatments increased the yield of maize in both soils in the order +P < +compost < +compost +P < +P-enriched compost. The maximum yields in the P-enriched compost treatment were nearly doubled in the German soil, but were increased more than 8-fold in the Pakistani soil. Root C in the fraction > 2 mm followed shoot C with a relatively stable shoot C-to-root C ratio of 4.5 in both soils. According to differences in $\delta^{13}C$ values, root material < 2 mm contributed 9% to the two treatments without compost amendment in the German soil and 11% in the Pakistani soil. In the German soil, 68% of the compost was recovered as particulate organic matter, in the Pakistani soil 64%. The microbial biomass-specific CO_2 evolution rate was much higher in the saline Pakistani soil. The absolute CO_2 evolution rate and also compost decomposition did not reveal strong differences between the two soils, contrasting the differences in maize yield and microbial biomass.

Key words: $\delta^{13}C$ • POM • Microbial biomass • Ergosterol • CO_2 evolution

Introduction

The fertility of Pakistani arable soils not only suffers from the severe hazard of salinity and alkalinity, they are also deficient in soil organic matter and endure the low availability of phosphorus, a major plant nutrient, as shown by the characterisation of 30 alkaline and saline arable soils from the Pakistani Punjab (Muhammad et al. 2005a). Biological amelioration of these soils is a most desirable aspect and the addition of compost is an important initiative in this direction. Biogenic household compost is an important component of waste management and might be an important source of macro and micronutrients, e.g. P and Zn (Niklasch and Joergensen 2001; Gattinger et al. 2004; Quintern et al. 2005). Research on the production and the use of compost has some tradition in the Punjab (Afghan et al. 1997; Bansal and Kapoor 1999).

However, our knowledge of interactions between plant growth and microbial decomposition of compost is very much restricted, due to severe methodological problems: (1) the measurements of microbial biomass and activity in the presence of living plants and (2) the differentiation between the growing plant and decomposing compost. However, the fumigation-extraction method with pre-extraction (Mueller et al. 1992; Mayer et al. 2003), especially in combination with ergosterol allows a reliable estimation of the microbial biomass in the presence of living roots (Joergensen 2000). The recovery of non-decomposed plant material by the simple sieving procedure of Magid and Kjaergaard (2001) and Magid et al. (2004) might be able to replace the litter-bag technique in arable soils (Muhammad et al. 2005b). Litter-bags have important drawbacks in monitoring decomposition processes by soil organisms, due to changes of

microclimate and especially due to a reduced contact between organic amendments and soil colloids (Fruit et al. 1999; Knacker et al. 2003). Progress in determining differences in the natural $\delta^{13}C$ signature of C3 and C4 plants (Balesdent and Mariotti 1996) enables us to differentiate between the roots of a C4 plant, such as maize, and decomposing C3 plant residues (Muhammad et al. 2005b). Maize is a very important crop in many regions and its large single plants leave a rather bare soil surface, enabling the measurement of CO_2 in pots by the dynamic chamber method (Wichern et al. 2004).

Under the arid or semiarid climate of Pakistan, salinity is usually combined with high pH conditions due to the presence and enrichment of calcium carbonate in the uppermost soil layers or to hydrolysis of sodium carbonate, leading to very low availability of P to plants (Muhammad et al. 2005a; Sandhu and Qureshi 1986). It was the aim of this study to investigate the interactions between plant growth, microbial biomass formation and compost decomposition. Therefore, an experiment was designed with different combinations of amending compost and triple superphosphate, comparing a strongly saline Pakistani arable soil with a non-saline German arable soil.

Material and Methods

Experimental procedure

The Pakistani arable soil included in the present study was collected from central Pakistan, in the region of Gujranwala, Punjab. Soil properties (Table 1) were determined as described by Muhammad et al. (2005a). In July 2002, the soil was sampled at 0-15 cm depth, sieved (< 2 mm) and transported to Witzenhausen, Germany. Similarly, an arable site from Frankenhausen, the experimental farm of the faculty, in

north Hessia, was sampled at 0-15 cm depth. About 100 kg of each soil was mixed with 100 kg of sterilised quartz sand (63–2000 µm mesh).

The experiment was carried out in a temperature-controlled greenhouse chamber at an average daily temperature of 22ºC with four replications and five treatments all with maize plant (*Zea mays* L.): (1) control, (2) +triple superphosphate, (3) +compost, (4) +compost +triple superphosphate, (5) +P-enriched compost. The different amendments were thoroughly mixed into the soil, transferred into pots (9.25 kg pot^{-1}) and adjusted to a bulk density of 1.4 g cm^{-3}. Triple superphosphate was applied at a rate of 200 µg P g^{-1} soil and compost at a rate of roughly 1%. The biogenic municipal waste compost was taken from the compost plant in the vicinity of Witzenhausen (Gattinger et al. 2004). The amount of compost was equivalent to 1.8 mg C g^{-1} soil, 175 µg N g^{-1} soil, and 67 µg P g^{-1} soil (Muhammad et al. 2005c). For the production of P-enriched compost, 100 g (on an oven-dry basis) biogenic municipal waste compost were thoroughly mixed with 1.85 g P as triple superphosphate and incubated for 24 h for application to the soil. Four seeds of maize (*Zea mays* L.) were sown at a depth of 3 cm. After emergence, two plants were screened out, so that each pot maintained two healthy plants up to maturity. The pots were placed in a completely randomized order on trays in the greenhouse. The moisture was kept at 30% of the water holding capacity by weighing twice a week and adding the water lost regularly. The experiment was started on 15 May 2004 and was carried out for 92 days until 15 August 2004.

Watering of the pots was stopped for 2 d before destructive sampling at the end of the experiment. This was carried out with two replicates of all treatments on two days. In each pot, shoots of the maize plants above the soil surface and also the roots growing through the bottom of the pots were removed, dried, weighed, and stored for further analysis. A soil sample of approximately 900 g was taken with a soil corer from the centre of each pot. After dry matter determination, the soil was sieved (< 2 mm) by

carefully crumbling it between the fingers to remove roots. Soil adhering to roots on top of the sieve were carefully removed from the roots and passed through the sieve. Separated roots were removed, dried, weighed, and stored for further analysis. The soil passed through the sieve was air-dried and stored for the analysis of particulate organic matter (POM), soil organic C, total N and $\delta^{13}C$.

Aliquots of the three organic amendments were dried at 60°C, ground in a Wiley mill to pass a 0.5-mm screen before elemental analysis (Table 2). Total C and total N were determined using a Vario Max CN analyser (Elementar, Hanau, Germany). Concentration of total P was determined after HNO_3/pressure digestion according to Heinrichs et al. (1986) as described by Chander et al. (2001) and analysed by ICP-AES (Spectro Analytical Instruments/Kleve).

Soil microbial properties

Microbial biomass C, biomass N and biomass P were estimated by the fumigation-extraction method (Brookes et al. 1982; Brookes et al. 1985; Vance et al. 1987) using the pre-extraction procedure of Mueller et al. (1992) as modified by Mayer et al. (2003).

For pre-extraction, 4 x 60 g soil (for microbial biomass C and biomass N) and 6 x 5 g soil (for microbial biomass P) were transferred into 250 ml or 100 ml plastic bottles and 150 ml or 50 ml demineralised water were added, respectively. The bottles were shaken for 20 minutes at 200 rev min^{-1}. The soil suspension was poured through a 2 mm sieve and 200 ml demineralised water were used to rinse roots and organic particles remaining on the sieve. Roots and organic particles were rejected. After stirring with a glass-stick, the suspension was allowed to sediment for at least 30 min. Roots and organic particles appearing at the surface of the suspension were removed using

tweezers. The suspension was poured into a folded filter paper (Schleicher & Schuell 595½, Dassel, Germany; 240 mm diameter for 60 g soil and 150 mm for 5 g soil).

For estimating microbial biomass C and biomass N, two of the pre-extracted 60-g portions were fumigated for 24 h at 25°C with ethanol-free $CHCl_3$. In addition to the usual fumigation procedure, 3 drops of liquid $CHCl_3$ were added directly to the soil samples in the filter paper. The fumigants were removed before the soil, including filter paper, was extracted with 200 ml 0.5 M K_2SO_4 by 30 min horizontal shaking at 200 rev min^{-1} and filtered (Schleicher & Schuell 595 ½). The non-fumigated portions plus filter paper were 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 E_C / k_{EC}, where E_C = (organic C extracted from fumigated soil) – (organic C extracted from non-fumigated soil) and k_{EC} = 0.45 (Wu et al. 1990, Joergensen 1996). Total N in the extracts was measured as activated NO_2^* by chemoluminescence detection (Dima-N, Dimatec) after combustion at 850°C. Microbial biomass N was E_N / k_{EN}, where E_N = (total N extracted from fumigated soil) - (total N extracted from non-fumigated soil) and k_{EN} = 0.54 (Brookes et al. 1985; Joergensen and Mueller 1996).

For estimating microbial biomass P, the first two 5-g portions were fumigated as described above, extracted with 100 ml 0.5 M $NaHCO_3$ (pH 8.5) by 30 min horizontal shaking at 200 rev min^{-1}, centrifuged for 15 min at (2000 *g*), and filtered (Schleicher & Schuell 595 ½). Two non-fumigated portions were extracted similarly at the time fumigation commenced. The remaining two portions were extracted after addition of 25 µg P g^{-1} (0.5 ml KH_2PO_4) in the same way as non-fumigated samples. Phosphate was measured by photo-spectrometry at 882 nm as described by Joergensen et al. (1995).

For the determination of ergosterol, 2 g moist soil was extracted with 100 ml ethanol for 30 minutes by horizontal shaking at 250 rev min^{-1} and filtered (Whatman

GF/A) (Djajakirana et al. 1996). Quantification was performed by reversed-phase HPLC at 26°C using a column of 125 x 4 mm Sphereclone 5µ ODS II with a guard column of 4 x 3 mm, 100% methanol as mobile phase, and 282 nm as resolution of detection.

Evolved CO_2 was measured once a week over a period of 2 minutes using a transportable infrared gas analyser with 2 to 5 replicates (Blanke 1996). The dynamic system consisted of a chamber (100 mm diameter, 150 mm height) coupled to a portable infrared gas analyser (IRGA) in a closed circuit (PP Systems, Hitchin, Herts., UK). The flow rate through the IRGA sensor cell during measurements was approximately 0.5 l min^{-1}. A small fan running at very low speed inside the chamber ensured that the air in the closed chamber was mixed during measurements. Soil temperature at 5 cm depth was measured concurrently using an attached temperature probe. Periodical measurements were taken between 10 a.m. and 1 p.m. The difference between the average temperature of the day and the temperature during the CO_2 measurements was corrected using the rate-modifying factor proposed by Jenkinson et al. (1987):

$$y = 47.9 / [1 + e^{106 / (x + 18.3)}] \quad (1)$$

The CO_2 evolution rate data in mg CO_2-C m^{-2} h^{-1} were taken as representative for half of the period between two measuring points and temperature was corrected according to the mean daily temperature during this interval. Finally, the CO_2 evolution rate was recalculated into µg CO_2-C g^{-1} soil for a specific interval or the whole experimental period.

Particulate organic matter and soil organic matter

Soil (400 g) was initially dispersed in 400 ml 5% NaCl, shaken by hand and allowed to stand for 45 min or over night. Then the samples were poured gradually onto sieves of 400 µm and subsequently 63 µm mesh sizes (Magid and Kjaergaard 2001; Magid et al. 2004) and washed with tap water. The aggregates were destroyed by pushing the soil through the sieve during the washing procedure until the water passing through the sieve became clear. The material retained on the sieve was transferred into a beaker. Tap water was added, the bucket was swirled and organic material was separated from the mineral material by flotation-decantation. Swirling and flotation-decantation was repeated several times, until organic particles were no longer visible in the mineral fraction. Then, the mineral fraction was discarded. The remaining two fractions of particulate organic matter (POM) 63 – 400 µm and POM > 400µm were transferred to a crucible, dried at 60°C, and ground for further analysis.

Total C and total N were determined gas-chromatographically after combustion using a Vario Max CN analyser (Elementar, Hanau) and $\delta^{13}C$ was measured on a Delta plus IRMS 251 (Finnigan Mat, Bremen) after combustion using a Carlo Erba NA 1500 gas chromatograph. Maize root derived C in the POM fractions was calculated from the $\delta^{13}C$ data by a modified form of the equation used by Balesdent and Mariotti (1996) according to Mueller et al. (1998):

$$C_{dfm} = C_{affected} \times [(\delta^{13}C_{affected} - \delta^{13}C_{non\text{-}affected}) / (\delta^{13}C_{maize} - \delta^{13}C_{non\text{-}affected})] \quad (2)$$

where C_{dfm} is C derived from maize roots in the two POM fractions, $\delta^{13}C_{affected}$ and $\delta^{13}C_{non\text{-}affected}$ are $\delta^{13}C(PDB)[‰]$ in the two POM fractions from the maize root affected or non-affected soil organic matter fractions, respectively. $\delta^{13}C_{maize}$ is $\delta^{13}C(PDB)[‰]$

measured in the maize root material. Non-affected $\delta^{13}C$ values were measured in the as initial values in the original soils without dilution with quartz sand.

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 experimental effects on microbial biomass indices was tested by a two-way ANOVA with soils and amendment treatments as main factors, followed by a soil effect-specific one-way analysis of variance using the Tukey/Kramer HSD-test (honestly significant difference). All statistical analyses were performed using StatView 5.0 (SAS Inst. Inc.).

Results

Microbial biomass indices

The saline Pakistani soil had a two times lower content of ergosterol, four times lower contents of microbial biomass C, biomass N and biomass P, but nearly a 20 times lower content of $NaHCO_3$ extractable P (Table 2). The microbial biomass C-to-N ratio and the ergosterol-to-microbial biomass C ratio were generally lower in the German soil than in the saline Pakistani soil (Table 3). The microbial biomass C-to-P ratios were similar in the control treatment of both soils, but always lower in the amendment treatments. The addition of 1% compost always had positive effects on the microbial properties analysed and also on the content of $NaHCO_3$ extractable P (Table 2). The absolute

increase in microbial biomass C and biomass N was larger in the German soil, the relative increase was larger in the saline Pakistani soil. The addition of compost led to increased microbial biomass C-to-N ratios, especially in combination with superphosphate (Table 3). The microbial biomass C-to-P was also increased by the compost amendment, but only without additional P fertilisation. The ergosterol-to-microbial biomass C ratio was generally lowered by the sole addition of compost but without soil-specific significance.

The addition of superphosphate solely or in either of the two combinations with compost did not have any marked effect on microbial biomass C and biomass N in both soils (Table 2). In contrast, superphosphate induced a strong and similar absolute increase in microbial biomass P in both soils, i.e. a stronger relative increase in the saline Pakistani soil. Maximum contents of microbial biomass P and $NaHCO_3$ extractable P were always measured in the P-enriched compost treatment, but the difference to the +compost +P treatment was not significant in most cases. Significant maximum contents of ergosterol were measured in the +compost +P treatment in both soils. In the +P treatment, the microbial biomass C-to-P ratio was strongly decreased compared to the control and the ergosterol-to-microbial biomass ratio was generally increased but without soil-specific significance (Table 3).

Maize growth

The yield in the non-amended control treatment of the German soil was more than 10 times larger than in the saline Pakistani soil (Table 4). The four different amendment treatments increased the yield of maize shoot C and shoot N per pot in both soils following the order +P < +compost < +compost +P < +P-enriched compost. Maximum

shoot-C and shoot-N yields in the +P-enriched compost treatment were nearly doubled in the German soil and were increased more than 8-fold in the saline Pakistani soil. The shoot C-to-N ratio was not significantly affected by any of the amendment treatments, but was 39 on average in the German soil and thus nearly double the average of 21 in the saline Pakistani soil.

Root C in the fraction > 2 mm followed shoot C with a relatively stable shoot C-to-root C ratio of 4.5 in both soils (Table 5). The soil-specific average $\delta^{13}C$ values of root C in the fraction > 2 mm were -15.1‰ in the German soil and -14.1‰ in the saline Pakistani soil, indicating the absence of significant percentages of compost C in this fraction. According to the $\delta^{13}C$ values of the POM fractions 63–400 µm and 400–2000 µm (Table 6), the contribution of maize roots could be calculated according to equation (2). Root material < 2 mm contributed 9% to the two treatments without compost amendment in the German soil and 11% in the Pakistani soil, with minor effects on the shoot C-to-root C ratio (Table 5).

Particulate organic matter and CO_2 evolution rate

The addition of compost significantly increased the two POM fractions 63–400 µm and 400–2000 µm (Table 6). However, this increase was not significant in the 63–400 µm fraction of the German soil, due to the 5 times larger background in the two treatments without compost amendment. Significantly lower $\delta^{13}C$ values in the POM fraction 400–2000 µm revealed the presence of maize root-derived organic matter in the treatments without compost amendment, comprising roughly 30% of this fraction in the German soil and 70% in the saline Pakistani soil.

The sum of CO_2-C evolved during the 92-day experimental period increased in the same order as the amount of shoot C and root C, i.e. control < +P < +compost < +compost +P < +P-enriched compost (Table 7). However, the difference between the control and +P treatment is not significant in either the soils for the total experimental period, but also for the three sub-periods. In the German soil, the three compost treatments led in most cases to significant increases in the CO_2 evolution rate compared to the other two treatments, but no significant differences occurred between the three compost treatments. In the saline Pakistani soil, the pure +compost treatment did not differ in any case significantly from the control treatments, although larger values were always reached. Compost-derived CO_2 was mainly evolved until 19 June, especially in the German soil, the root-derived CO_2 from 28 July until the end of the experiment (Table 7, Fig. 1a/b).

Table 1. Properties of the Pakistani soil and the German soil used in the 92-day greenhouse experiment without dilution with quartz sand

Properties	German soil	Pakistani soil
Sand (%)	11.5	25
Silt (%)	76.5	65
Clay (%)	14.5	10
$CaCO_3$ (%)	3	16.1
pH-H_2O	7.4	8.2
Salt (mg g^{-1} soil)	0	6.0
Salt-Na (mg g^{-1} soil)	0	1.45
Salt-HCO_3 (mg g^{-1} soil)	0	0.10
Soil organic C (mg g^{-1} soil)	13	5.4
Total N (mg g^{-1} soil)	1.4	0.55
Total P (mg g^{-1} soil)	0.99	0.67

Table 2. Contents of microbial biomass indices and 0.5 M $NaHCO_3$ extractable P at the end of the 92-day greenhouse experiment

	Microbial biomass C ($\mu g\ g^{-1}$ soil)	Microbial biomass N ($\mu g\ g^{-1}$ soil)	Microbial biomass P ($\mu g\ g^{-1}$ soil)	0.5 M $NaHCO_3$ extractable P ($\mu g\ g^{-1}$ soil)	Ergosterol ($\mu g\ g^{-1}$ soil)
German soil					
Control	196 a	38.5 a	20.1 a	34.1 a	0.57 a
+P	171 a	25.1 a	28.9 b	42.1 bc	0.77 a
+Compost	387 b	56.2 b	23.0 ab	40.6 b	0.91 a
+Compost +P	455 b	62.1 b	36.2 c	46.4 c	1.88 b
+P-enriched compost	387 b	52.8 ab	40.8 c	47.4 c	0.69 a
Pakistani soil					
Control	48 a	7.2 a	5.2 a	1.8 a	0.26 a
+P	41 a	5.1 a	18.3 bc	9.6 b	0.29 a
+Compost	151 b	21.6 c	14.4 b	9.3 b	0.51 a
+Compost +P	156 b	17.2 b	22.1 cd	17.8 c	0.84 b
+P-enriched compost	152 b	20.3 bc	25.8 d	20.2 d	0.51 a
Analysis of variance					
Soils	<0.001	<0.001	<0.001	<0.001	<0.001
Amendments	<0.001	<0.001	<0.001	<0.001	<0.001
S x **A**	<0.001	<0.001	0.115	0.011	0.110
CV (±%)	12	14	10	9	20

Different letters within a soil-specific column indicate a significant difference ($P < 0.05$, Tukey/Kramer, n = 4); CV = mean coefficient of variation between replicate pots

Table 3. The ratios microbial biomass C-to-N, biomass C-to-P, and ergosterol-to-microbial biomass C at the end of the 92-day greenhouse experiment

Treatments	Microbial biomass C/N	Microbial biomass C/P	Ergosterol/microbial biomass C (%)
German soil			
Control	5.1 a	9.8 b	0.30 ab
+P	6.8 b	5.9 a	0.46 b
+Compost	6.9 b	16.9 c	0.24 a
+Compost +P	7.3 b	12.7 b	0.41 b
+P-enriched compost	7.4 b	9.5 b	0.18 a
Pakistani soil			
Control	6.7 a	9.3 b	0.62 a
+P	7.9 a	2.3 a	0.75 a
+Compost	7.0 a	11.1 b	0.34 a
+Compost +P	9.3 a	7.2 ab	0.54 a
+P-enriched compost	7.5 a	6.0 ab	0.34 a
Analysis of variance			
Soils	0.006	<0.001	0.001
Amendments	0.001	<0.001	0.003
S x **A**	0.264	0.106	0.666
CV (±%)	7	8	14

Different letters within a soil-specific column indicate a significant difference ($P < 0.05$, Tukey/Kramer, n = 4); CV = mean coefficient of variation between replicate pots

Table 4. Organic C, total N, and total P, the ratios organic C-to-total N and organic C-to-total P in maize shoots at the end of the 92-day greenhouse experiment

Treatments	Shoot-C (g pot^{-1})	Shoot-N (g pot^{-1})	Shoot-P (mg pot^{-1})	Shoot-C/N	Shoot-C/P
German soil					
Control	23.3 a	0.60 a	103 a	42 a	228 a
+P	24.0 a	0.61 a	109 a	43 a	225 a
+Compost	29.7 ab	0.81 ab	137 a	38 a	217 a
+Compost +P	34.1 b	0.98 ab	152 ab	35 a	224 a
+P-enriched compost	43.7 c	1.20 b	198 b	37 a	223 a
Pakistani soil					
Control	2.1 a	0.11 a	7 a	20 a	335 a
+P	5.9 b	0.25 b	25 b	25 a	238 a
+Compost	8.6 c	0.43 c	21 b	20 a	505 a
+Compost +P	11.4 d	0.59 d	45 c	19 a	254 a
+P-enriched compost	17.2 e	0.82 e	65 d	22 a	268 a
Analysis of variance					
Soils	<0.001	<0.001	<0.001	<0.001	0.008
Amendments	<0.001	<0.001	<0.001	0.346	0.118
S x **A**	0.140	0.901	0.071	0.837	0.088
CV (±%)	3	18	21	10	18

Different letters within a soil-specific column indicate a significant difference ($P < 0.05$, Tukey/Kramer, n = 4); CV = mean coefficient of variation between replicate pots

Table 5. Organic C contents and $\delta^{13}C$ in maize roots and the shoot C-to-root C ratio of the maize plants at the end of the 92-day greenhouse experiment; maize root C < 2 mm according to the $\delta^{13}C$ values in Table 6

	Root C > 2 mm (+ < 2 mm) (g pot^{-1})	Root $\delta^{13}C$ (δ‰)	Shoot C/root C > 2 mm (+ < 2 mm)
German soil			
Control	4.5 a (5.0)	-14.7 a	5.4 ab (4.7)
+P	4.2 a (4.6)	-15.1 a	5.8 b (5.3)
+Compost	6.8 b	-15.6 a	4.4 ab
+Compost +P	10.3 b	-14.9 a	3.3 a
+P-enriched compost	10.9 b	-15.3 a	4.0 ab
Pakistani soil			
Control	0.5 a (0.6)	-12.8 a	3.9 a (3.2)
+P	1.2 ab (1.4)	-13.2 ab	5.4 a (4.4)
+Compost	1.9 b	-15.3 b	4.8 a
+Compost +P	3.3 c	-15.1 b	3.7 a
+P-enriched compost	4.3 c	-13.9 ab	4.0 a
Analysis of variance			
Soils	<0.001	<0.001	0.554
Amendments	<0.001	0.002	0.037
S x **A**	<0.001	0.039	0.571
CV (±%)	4	2	6

Different letters in a soil-specific row show significant difference ($P < 0.05$, Tukey/Kramer, n = 4); CV = mean coefficient of variation between replicate pots

Table 6. Organic C contents and $\delta^{13}C$ values in the two particulate organic matter (POM) fractions at the end of the 92-day greenhouse experiment; in brackets maize-root derived C according to the $\delta^{13}C$ values, initial values were measured in the original soils without dilution with quartz sand

	POM-C 400–2000 µm (µg g^{-1} soil)	POM-C 63–400 µm (µg g^{-1} soil)	POM-$\delta^{13}C$ 400–2000 µm (δ‰)	POM-$\delta^{13}C$ 63–400 µm (δ‰)
German soil				
Initial values			-27.8 c	-27.2 a
Control	227 a (58)	678 a	-24.6 ab	-26.5 a
+P	107 a (34)	710 a	-23.8 a	-26.0 a
+Compost	1046 b	948 a	-26.2 bc	-26.8 a
+Compost +P	1037 b	1174 a	-26.2 bc	-26.7 a
+P-enriched compost	1131 b	944 a	-26.4 c	-26.4 a
Pakistani soil				
Initial values			-24.0 b	-22.1 a
Control	13 a (9)	161 a	-17.5 a	-23.7 b
+P	18 b (13)	128 a	-16.6 a	-22.0 a
+Compost	836 c	369 b	-22.8 b	-26.7 c
+Compost +P	868 c	417 b	-23.9 b	-26.6 c
+P-enriched compost	876 c	561 b	-23.9 b	-26.5 c
Analysis of variance				
Soils	0.009	<0.001	<0.001	<0.001
Amendments	<0.001	0.001	<0.001	<0.001
S x **A**	0.941	0.527	<0.001	<0.001
CV (±%)	10	14	2	5

Different letters within a soil-specific column indicate a significant difference ($P < 0.05$, Tukey/Kramer, n = 4); CV = mean coefficient of variation between replicate pots

Table 7. CO_2-C evolved during the complete 92-day greenhouse experiment and during 3 different sub-periods

Treatments	ΣCO_2-C 16.5.–19.6. 35 d ($\mu g\ g^{-1}$ soil)	ΣCO_2-C 20.6–27.7. 38 d ($\mu g\ g^{-1}$ soil)	ΣCO_2-C 28.7.–15.8. 19 d ($\mu g\ g^{-1}$ soil)	ΣCO_2-C 16.5.–15.8. 92 d ($\mu g\ g^{-1}$ soil)
German soil				
Control	99 a	93 a	301 a	493 a
+ P	102 a	97 a	396 ab	595 a
+ Compost	421 b	278 b	790 bc	1489 b
+ Compost + P	448 b	252 b	968 c	1668 b
+ P-enriched compost	475 b	155 a	1104 c	1734 b
Pakistani soil				
Control	192 a	56 a	288 a	536 a
+ P	200 a	82 ab	264 a	546 a
+ Compost	233 a	135 ab	379 a	747 ab
+ Compost +	406 a	148 b	623 ab	1177 bc
+ P-enriched compost	467 a	137 ab	926 b	1530 c
Analysis of variance				
Soils	0.868	<0.001	0.004	0.001
Amendments	0.001	<0.001	<0.001	<0.001
S x **A**	0.486	0.003	0.372	0.029
CV (±%)	10	8	10	7

Different letters within a soil-specific column indicate a significant difference ($P < 0.05$, Tukey/Kramer, n = 4); CV = mean coefficient of variation between replicate pots

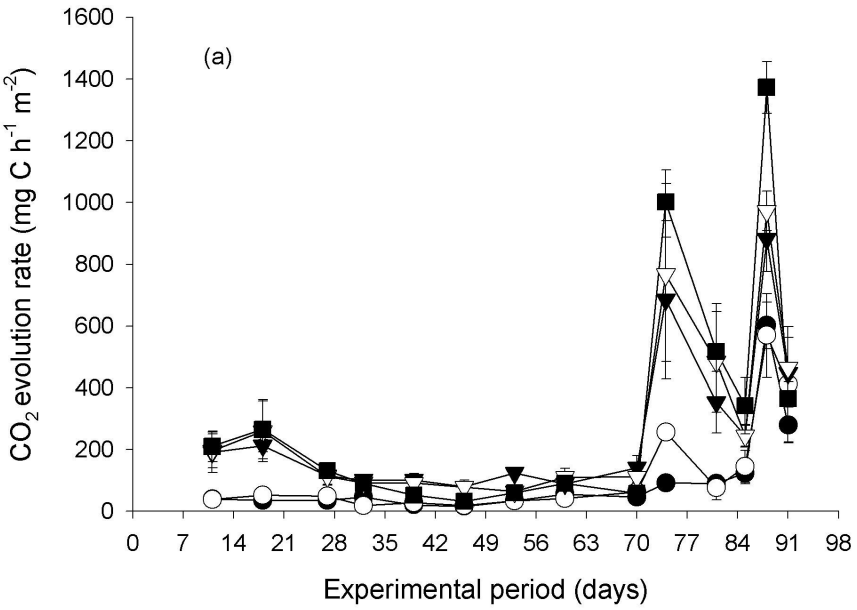

Fig. 1. CO_2 evolution rates in the different treatments of the (a) German soil and (b) saline Pakistani soil during the 92-day greenhouse experiment at 22°C average daily temperature from 15 May to 15 August 2004; control (●), +P (○), +compost (▼), +compost +P (▽), and P-enriched compost (■)

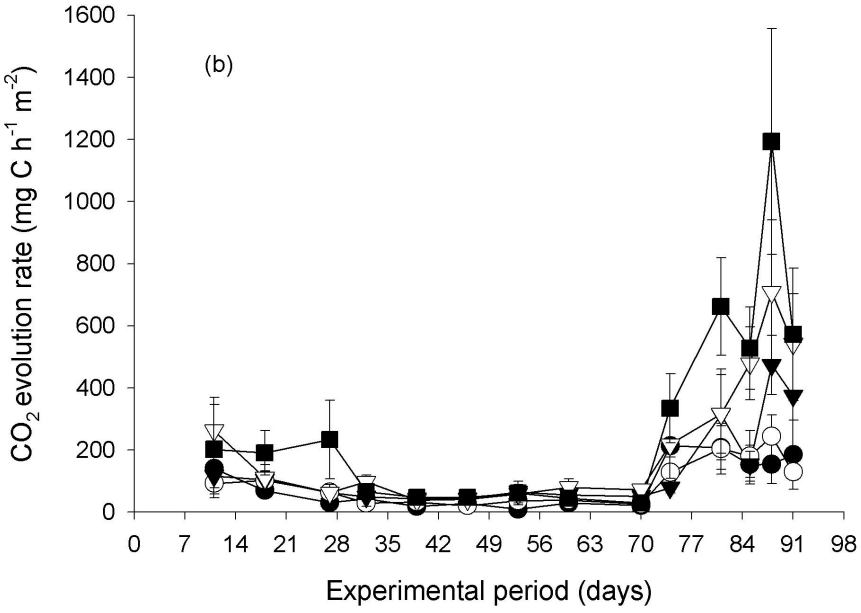

Fig. 2. Compost organic C recovered at the end of the 92-day greenhouse experiment at 22°C average daily temperature from 15 May to 15 August 2004 as microbial biomass C and as particulate organic matter (POM) in the fraction < 2 mm (= 63–400 µm + 400–2000 µm)

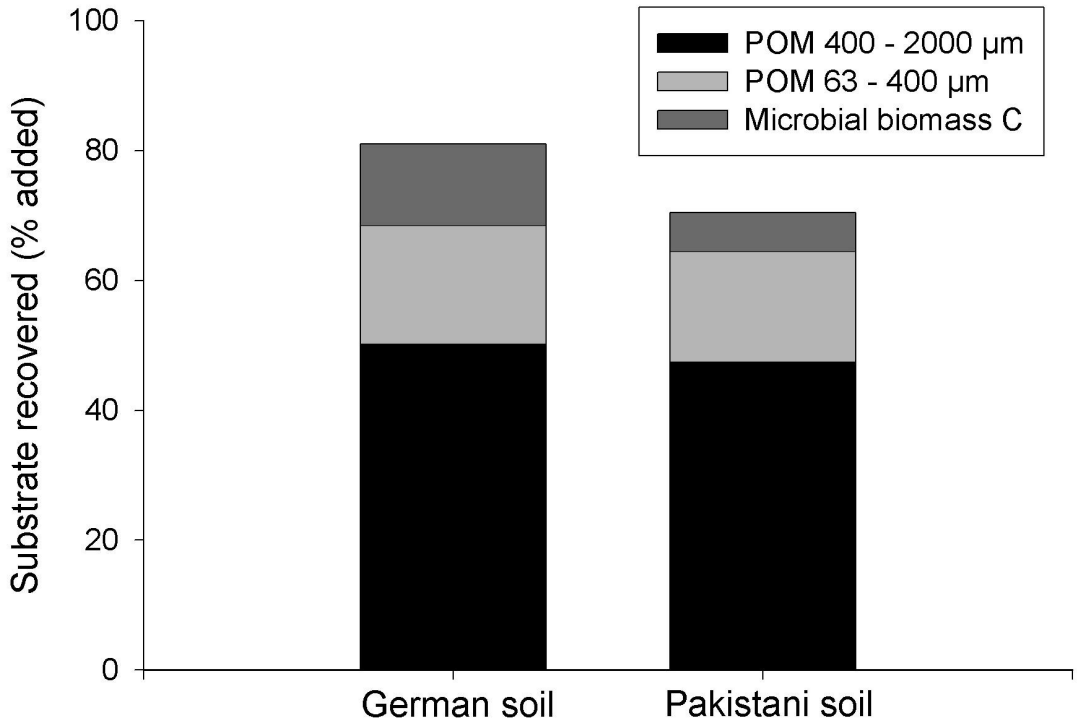

Fig. 3. Organic C recovered at the end of the 92-day greenhouse experiment 22°C average daily temperature from 15 May to 15 August 2004 as particulate organic matter (POM) in the fractions 63 – 400 µm and 400 – 2000 µm, and as microbial biomass C

Discussion

The response of soil microbial biomass C and biomass N to the four different amendments formed just two groups, one with compost addition and the other without. The formation of these two levels within a certain range of C input by maize roots again supports the hypothesis of a biological space in soil, maintaining very stable amounts of microbial biomass within a wide range of organic matter input (Nannipieri et al. 1978). However, above a certain input threshold induced by compost, the soil microbial indices were significantly increased, demonstrating the importance of compost for improving the biological soil quality (Banger et al. 1989; Niklasch and Joergensen 2001; Quintern et al. 2005). A certain amount of microorganisms is directly added by the compost (Gattinger et al. 2004). Nevertheless, the increase in microbial biomass was four times higher in the German soil and two times higher in the saline Pakistani soil than the amount added directly with the compost (Muhammad et al. 2005c). The differences in net increase between the soils may be caused by an initially much lower microbial biomass content in the saline Pakistani soil. It has been shown repeatedly that the net increase in microbial biomass depends on the ratio of substrate to the initial microbial biomass, i.e. the lower the microbial biomass the smaller the net increase (Witter and Kanal 1998; Chander and Joergensen 2001). The relatively high CO_2 evolution rates in the Pakistani soil strongly indicate that the lower increase in microbial biomass is mainly due to a considerably lower substrate use efficiency of the microbial community in this saline soil.

Not only were the formation and maintenance of microbial biomass affected in the saline Pakistani soil, but also the yield and growth characteristics of maize, which is not a species very sensitive to salinity (Jumberi et al. 2001). The much lower yield of the maize plants was expected in the saline Pakistani soil, also the stronger relative

response to the amendments in comparison to the German soil (Banger et al. 1989; Ben-Hur et al. 2001; Manna et al. 2001). A decrease in shoot N contents of barley has been reported with increasing salinity (Ali et al. 2001), but no information is available on a salinity-induced decrease in shoot C-to-N ratios of maize, which was found in all five treatments of the Pakistani soils. This clearly indicates that nutrient deficiency could not have been the reason for the decreased shoot C-to-N level, especially considering that the biogenic compost contains a large variety of all necessary macro and micronutrients (Gattinger et al. 2004; Quintern et al. 2005).

In contrast to microbial biomass C and biomass N, shoot and root biomass as plant growth indices revealed strong differences between the five treatments in their ability to supply nutrients to maize. The differences between the two compost treatments with addition of easily soluble P in the form of superphosphate are extraordinary considering the fact that there was no difference in the total amount of nutrients added. A 24 h pre-incubation period of compost with superphosphate and the following combined amendment of the two components enormously increased the availability of the added P to the plant and also led to maximum microbial biomass P contents. The reason for this increased bioavailability is most likely the immediate uptake of the added phosphorus by compost-colonising microorganisms followed by a slow release during the experimental period. However, it is not known at present whether this extra phosphorus comes from the mineralization of dead microorganisms or a slow excretion by the soil microbial biomass, which would consequently increase the microbial biomass C-to-P ratio.

The microbial biomass apparently has an important sink and source function for phosphorus. Approximately 2% of added phosphorus was taken up by the maize plants in the +P treatment, 4% remained in a $NaHCO_3$ extractable form, but 5% of added P was incorporated into the microbial biomass. The percentage of microbial incorporation

of added P was similar after compost or superphosphate amendment, indicating the importance of soil biological processes for the nutrition of plants with P from inorganic or organic sources. On the other hand, differences in P availability have marked effects on the microbial community structure as demonstrated by the strong increase in ergosterol content in the compost +P treatment, indicating a shift towards fungi. In contrast to previous observations, the ergosterol content was consistently higher in the saline Pakistani soil than in the German soil for unknown reasons (Muhammad et al. 2005b/c). Maize growth and dilution of the soil with sterile quartz sand might have contributed to the relatively large ergosterol content in the saline Pakistani soil. However, it is known from a previous survey of 30 alkaline and saline Pakistani soils that the ergosterol-to-microbial ratio is able to reach a maximum value of 1% (Muhammad et al. 2005b), much higher than in most German arable soils (Djajakirana et al. 1996).

Assuming that neither the addition of superphosphate nor the compost amendment affected the decomposition of native POM, the percentage of compost C recovered could be calculated as follows:

$$\text{POM-C}_{\text{compost}} = (\text{POM-C}_{\text{compost-amendment}} - \text{POM-C}_{\text{no-compost-amendment}}) \text{ / compost-C x } 100,$$

where $\text{POM-C}_{\text{compost}}$ is the percentage of compost C recovered as POM, $\text{POM-C}_{\text{compost-amendment}}$ is the average amount of POM-C in the treatments +compost, + compost +P, and +P-enriched compost, and $\text{POM-C}_{\text{no-compost-amendment}}$ is the average amount of POM-C in the control and +P treatment (Fig. 2). On the basis of this calculation, 68% was recovered as particulate organic matter in the German soil and 64% in the saline Pakistani soil.

Assuming that the net increase in microbial biomass C in the three compost treatments was exclusively caused by the decomposition of added compost, 13% of this substrate was incorporated as microbial biomass C in the German soil and 6% in the saline Pakistani soil. Then, it can be concluded from Fig. 2 that 19% and 28% of the compost C added can be attributed to CO_2 and to microbial residues in the German soil or in the saline Pakistani soil, respectively. Assuming soil-specific constant ratios of CO_2-C-to-root C in all treatments, the 19% would be consistent with the CO_2 evolution rates measured in the German soil, but without leaving a balance gap for the formation of microbial residues. This would be in accordance with the incubation experiment using the same biogenic compost described by Muhammad et al. (2005c). In the saline Pakistani soil, lower CO_2 evolution rates were measured in the three compost treatments, which is in contrast to the lower recovery of compost as POM. However, the CO_2 evolution data were measured only once a week and showed strong variation between the replicates. In contrast to the experiment with alfalfa residues (Muhammad et al. 2005b), the general level of the additional CO_2 evolution rate in the compost treatments is in line with the CO_2 production rates in incubation experiments (Muhammad et al. 2005c). The mineralization rates in the present compost treatments were in the range of C-specific CO_2 production rates of biogenic municipal waste composts at different maturity stages (Gattinger et al. 2004) and also similar to that observed by Rasul et al. (2005) who measured a 20% loss of CO_2-C in a treatment with biogenic municipal waste compost from the same compost plant at a 1% addition rate during a 63-day incubation at 25°C. In contrast to the plant yield and the formation of microbial biomass, the present experiment did not reveal any clear indications that mineralization of added compost was strongly reduced by salinity.

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6. Conclusions

These studies were related to organic substrate-soil interactions and were aimed at establishing appropriate methods for enhancing soil fertility, organic matter and microbial biomass in depleted saline and alkaline soils of Pakistan. The examined organic substrate incorporation techniques, in growing maize crop in pots or directly incorporated in saline and alkaline soils in incubation experiments, provided us sound evidence that addition of substrates under alkaline and saline conditions has stronger effects on microbial biomass, but only slight effects on the mineralization of added substrates. This provides relatively simple and suitable methodology for estimating the effects of incorporated organic substrates on microbial biomass dynamics. Therefore, it has advantages in estimating effects of added organic amendments on the soil microbial community, soil organic matter (SOM), and root and substrate interaction and decomposition rate effect simultaneously. However, this approach may not account for certain biological complications.

The present study showed that neither the addition of alfalfa residues nor the growth of maize plants alone lead to significant changes in any of the soil microbial properties analysed in comparison to the control soil. In contrast to this, the combination of growing maize plants and alfalfa residues increased the contents, in most cases significantly, of microbial biomass C (36%), biomass N (28%), biomass P (15%) and ergosterol (24%). These differences in increases led to a significant increase in the microbial biomass C-to-N ratio, but the microbial biomass C-to-P ratio and the ergosterol-to-microbial biomass C ratio remained unchanged. The amount of the maize shoot C was more than doubled in treatment 4 with alfalfa residues than without in treatment 3. In these two maize growth treatments, the mean shoot C-to-N ratio was 90 and the mean shoot C-to-root C ratio 5.3, without any significant treatment effects.

Plant remains > 2 mm increased significantly in the order alfalfa residues < maize roots < maize roots plus alfalfa residues. Only 6% of the alfalfa residues could be recovered as plant remains > 2 mm in treatment 2. The two POM fractions 63–400 µm and 400–2000 µm comprised 10% and 4% of soil organic C. Only minor amounts of maize roots and alfalfa residues were transferred into these two fractions according to the non-significant differences between the three amendment treatments and the control. The differences were small in most cases except treatment 4 with maize growth plus alfalfa residues. The $\delta^{13}C$ value of recovered alfalfa residues > 2 mm was lowest at -28.2 δ‰ and that of maize roots was highest at -14.9 δ‰. Based on these significantly differing values, the contribution of these two sources to the fraction of plant remains > 2 mm could be calculated according to equation (2). Maize roots contributed 836 µg C g^{-1} soil to this fraction and alfalfa residues 144 µg C g^{-1} soil, equivalent to 85.3% and 14.7%, respectively. This means that 54 µg g^{-1} soil or 60% more alfalfa-C were recovered in treatment 4 than in treatment 2. The significantly higher $\delta^{13}C$ values in the POM fractions 63–400 µm and 400–2000 µm of treatment 4 in comparison to the control treatment revealed the occurrence of 63 µg maize root C g^{-1} soil in both fractions, equivalent to 4.6% and 14% of the two POM fractions.

In treatments 2 and 3, i.e. the sole alfalfa residues and sole maize growth treatments, the sum of CO_2-C evolved during the 90 day pot experiment was on the same level and nearly doubled in comparison to the control soil. The sum of CO_2-C evolved in the maize plus alfalfa treatment was roughly 60% larger, i.e. the amount of substrate-derived CO_2-C of this treatment was twice that of the sole maize growth treatments. In treatment 2, the sum of substrate-derived CO_2-C was equivalent to 18% of the added alfalfa residue C. From 11 June (6 days after sowing) until 2 July, the CO_2 evolution rate of the two maize treatments was higher than that of both the sole alfalfa residues treatment and the control soil. From 3 July until the end of the experiment, the

CO_2 evolution rate of the sole alfalfa residues treatment was only slightly above that of the control soil. Only 11% of the difference between these two treatments was produced during the last 48 days of the experiment.

The level of soil organic matter and microbial biomass indices was generally very low in the saline and alkaline soils from 30 Pakistani arable sites, but no strong direct effects of salinity and alkalinity on microbial biomass indices could be observed. The microbial biomass C-to-soil organic C ratio was within range of arable soils from a temperate humid climate at neutral pH, indicating that saline and alkaline soil conditions did not reduce the accessibility of organic matter to soil micro-organisms. Only basal respiration was negatively affected by salinity indices, but not by soil pH. High levels of the metabolic quotient qCO_2 indicate that the microorganisms are generally stressed by the saline and alkaline conditions, leading to low substrate use efficiencies. This intensifies the threat of further losses in soil fertility by the reduction in soil organic matter levels, which is emphasized by the classical negative relationship between qCO_2 and the microbial biomass C-to-soil organic C ratio. Also the microbial community structure was not directly affected by salinity according to the gross ratios microbial biomass C-to-N, microbial biomass C-to-P, and also ergosterol-to-microbial biomass C. Only an increasing pH at alkaline conditions has some negative effects on ergosterol contents in soil and thus on soil microfungi.

Salinity under alkaline conditions has strong effects on the incorporation of organic amendments into the microbial biomass, but only slight effects on the mineralization of compost, maize straw or pea straw to CO_2. Both processes are not linearly related to increasing salinity. Salt effects on soil biological properties are significant only above a threshold of 10 mS cm^{-1} electrical conductivity or a salt content of 3 mg g^{-1} soil. The salinity effects on mineralization of maize straw or pea straw to CO_2 were stronger than those on compost. The combined observation of the decomposition process by CO_2

evolution rate and the recovery of unused substrate as POM are useful and make it possible to calculate yield coefficients for complex organic substrates.

Addition of compost and /or phosphate (mineral) fertiliser under growing maize crop enhanced the growth of maize crop and soil biological parameters alike. This change occurred in both German and Pakistani soils. We found that P-enriched compost enhanced microbial biomass P and extractable-P efficiently, and compost + P increased microbial biomass C. But this increase was equal in microbial biomass N in all three treatments with compost or compost with mineral fertiliser in both German and Pakistani soils. The maximum increase in fungal biomass was observed in compost + P added treatment in both soils. Organic C and N from particulate organic matter (POM) increased significantly in 3, 4, and 5 treatments. These results revealed that addition of compost increased organic C and N in the residues over the control and only + P added treatments. Similarly, maize crop also enriched $\delta^{13}C$ as indicated by POM. In short, we can say that addition of compost increased organic matter in the soil and ultimately enhanced soil microbial biomass, mobilised nutrients and increased their availability in the root zone for plant growth.

7. Summary

7.1 Impact of growing maize (*Zea mays*) on the decomposition of incorporated fresh alfalfa residues

In this study, the effects of growing maize plants on the microbial decomposition of easily degradable plant residues was investigated in a 90-day pot experiment using a sandy arable soil. Four treatments were carried out: (1) untreated control, (2) with freshly chopped alfalfa residues (*Medicago sativa* L.) incorporated into soil, (3) with growing maize plants (*Zea mays* L.), and (4) with growing maize plants and freshly chopped alfalfa residues incorporated into soil. The amount of alfalfa residues was equivalent to 1.5 mg C g^{-1} soil and 120 µg N g^{-1} soil. Only the combination of growing maize plants and alfalfa residues significantly increased the contents of microbial biomass C, microbial biomass N, and ergosterol in soil compared to the control. The dry weight of the maize shoot material was more than doubled in the treatment with alfalfa residues than without. Assuming that the addition of alfalfa residues did not affect the decomposition of native soil organic matter, only 27% of the alfalfa residues were found as CO_2 using a portable gas analyser with a dynamic chamber. This suggests that a considerable part of alfalfa-C remained undecomposed in the soil. However, only 6% of the alfalfa residues could be recovered as plant remains > 2 mm in treatment 2. The reasons for this discrepancy are discussed. In the particulate organic matter (POM) fractions 63–400 µm and 400–2000 µm there are no indications that any alfalfa residues were transferred into this fraction. Based on $\delta^{13}C$ values, it was calculated that plant remains > 2 mm in treatment 4 contained 14.7% alfalfa residues and 85.3% maize root remains. This means that 54 µg g^{-1} soil or 60% more alfalfa-C was recovered in this treatment than in treatment 2. The reasons for this apparent retardation in the

decomposition of alfalfa residues and the problems of the methodological approach are discussed.

7.2 Relationships between soil biological and other soil properties in saline and alkaline arable soils from the Pakistani Punjab

The interactions between soil physical, soil chemical and soil biological properties were analysed in 30 Pakistani soils from alkaline and saline arable sites differing strongly in salinisation and in soil pH. The soil biological properties were differentiated into indices for microbial activity (basal respiration), microbial biomass (C, N, and P), and community structure (fungal ergosterol) with the aim of assessing their potential as soil fertility indices in alkaline and saline arable soils. All soils were salt affected, mainly by Na^+ and HCO_3^-, and their texture was dominated by silt. The median contents of soil organic C, total N and total P were 4.5 mg g^{-1} soil, 0.45 mg g^{-1} soil, and 0.66 mg g^{-1} soil, respectively. On average, only 1.0% of total P was extractable by 0.5 M $NaHCO_3$. The median contents of microbial biomass C, biomass N, biomass P, and ergosterol were 94 µg g^{-1} soil, 11 µg g^{-1} soil, 4.6 µg g^{-1} soil, and 0.16 µg g^{-1} soil, respectively. Basal respiration varied around a median of 8.6 µg CO_2-C d^{-1} g^{-1} soil and was the only soil biological property negatively affected by increasing salinity. Ergosterol was negatively related to soil pH. The microbial biomass C-to-P ratio exceeded the soil organic C-to-total P ratio nearly threefold, but the soil organic C-to-total N ratio and microbial biomass C-to-N ratio were both at a similar level around 9.0. For this reason, the microbial biomass C-to-soil organic C ratio and the microbial biomass N-to-total N ratio were also both at a similar level at approximately 2.2%. The microbial biomass P-total P ratio was three times smaller. Salt-derived Na also had some negative effects on

the metabolic quotient qCO_2. High levels of the metabolic quotient qCO_2 indicate that the microorganisms are generally stressed by the saline and alkaline conditions, leading to low substrate use efficiencies. This intensifies the threat of further losses in soil fertility by the reduction in soil organic matter levels.

7.3 Decomposition of compost and plant residues in Pakistani soils along a gradient in salinity

Three organic amendments (compost, maize straw and pea straw) were added to five Pakistani soils from a gradient in salinity to test the following two hypotheses: (1) Increasing salinity at high pH decreases proportionally the decomposition of the added organic amendments and the resulting net increase in microbial biomass; (2) salinity effects override differences in quality of the organic amendments. Although salinity has depressive effects on microbial biomass C, biomass N, biomass P, and ergosterol, the clear gradient according to the soil salt concentration was not reflected by the soil microbial properties. Nevertheless, the ratios microbial biomass C-to-N and biomass C-to-P decreased continuously with increasing salinity. In contrast, the ergosterol-to-microbial biomass C ratio was constant in the four soils at pH > 8.9, but nearly doubled in the most saline, but least alkaline soil. The addition of the three organic amendments always increased the contents of the microbial indices analysed. The amendment-induced increase was especially strong for microbial biomass P and reflected the total P content of the added substrates. Highest sampling date-specific mean contents of microbial biomass C and biomass N were measured at day 0, immediately after the amendments were added. The organic amendments increased the CO_2 evolution rates of all 5 soils in a soil-specific and amendment-specific way, but without a clear effect of

salinity. The same was true for total C and total N in the two fractions of particulate organic matter (POM) 63 – 400 µm and > 400 µm. Lowest percentage of substrate derived CO_2-C-and highest recovery of POM-C was observed in the compost treatment and the reverse in the pea straw treatment. The percentage of N recovered as POM was much smaller than the percentage of C in the compost and in the pea straw treatment, but not in the maize straw treatment.

7.4 Interactions of compost and triple superphosphate on the growth of maize (*Zea mays*) in a saline Pakistani soil

A greenhouse pot experiment was designed with different combinations of compost and triple superphosphate amendments to investigate the interactions between plant growth, microbial biomass formation and compost decomposition in a strongly saline Pakistani arable soil in comparison to a non-saline German arable soil. The Pakistani soil had a two times lower content of ergosterol, four times lower contents of microbial biomass C, biomass N and biomass P, but nearly a 20 times lower content of $NaHCO_3$ extractable P. The addition of 1% compost always had positive effects on the microbial properties and also on the content of $NaHCO_3$ extractable P. The addition of superphosphate solely or in the treatments +compost +P or +P-enriched compost induced a strong and similar absolute increase in microbial biomass P in both soils. The yield in the control treatment of the German soil was more than 10 times larger than in the Pakistani soil. The amendment treatments increased the yield of maize in both soils in the order +P < +compost < +compost +P < +P-enriched compost. The maximum yields in the P-enriched compost treatment were nearly doubled in the German soil, but were increased more than 8-fold in the Pakistani soil. Root C in the fraction > 2 mm

followed shoot C with a relatively stable shoot C-to-root C ratio of 4.5 in both soils. According to differences in $\delta^{13}C$ values, root material < 2 mm contributed 9% to the two treatments without compost amendment in the German soil and 11% in the Pakistani soil. In the German soil, 68% of the compost was recovered as particulate organic matter, in the Pakistani soil 64%. The microbial biomass-specific CO_2 evolution rate was much higher in the saline Pakistani soil. The absolute CO_2 evolution rate and also compost decomposition did not reveal strong differences between the two soils, contrasting the differences in maize yield and microbial biomass.

8. Zusammenfassung

8.1 Einfluss von wachsendem Mais (*Zea mays*) auf die Zersetzung von eingearbeiteten frischen Luzernerückständen

In dieser Studie wurde der Einfluss von wachsenden Maispflanzen auf die mikrobielle Zersetzung von leicht abbaubaren Pflanzenrückständen in einem 90-tägigen Gefäßversuch an einem sandigen Ackerboden untersucht. Vier Varianten wurden durchgeführt: (1) unbehandelte Kontrolle, (2) mit frisch zerkleinerten und in den Boden eingemischten Luzernerückständen (*Medicago sativa* L.), (3) mit wachsenden Maispflanzen (*Zea mays* L.), und (4) mit wachsenden Maispflanzen und frisch zerkleinerten und in den Boden eingemischten Luzernerückständen. Die Menge an Luzernerückständen entsprach 1.5 mg C g^{-1} Boden und 120 µg N g^{-1} Boden. Nur in der Kombination von wachsenden Maispflanzen und Luzernerückständen wurden die Gehalte an mikrobiellem Biomasse-C, mikrobiellem Biomasse-N und Ergosterol im Boden im Vergleich zur Kontrolle signifikant erhöht. Die Trockenmasse von Maissprossmaterial wurde in der Variante mit Luzernerückständen im Vergleich zur Variante ohne mehr als verdoppelt. Unter der Annahme, dass die Zugabe von Luzernerückständen keinen Einfluss auf die Zersetzung von nativer organischer Bodensubstanz hat, konnten nur 27% der Luzernerückstände als CO_2, wieder gefunden werden, gemessen mit einem tragbaren Gasanalysator mit dynamischer Messkammer. Dieses weist darauf hin, dass eine beträchtliche Menge an Luzerne-C unzersetzt im Boden verblieben ist. Jedoch konnten nur 6% der Luzernerückstände als Pflanzenreste > 2 mm in der Variante 2 wiedergewonnen werden. Die Gründe für diesen Unterschied wurden diskutiert. In den Fraktionen der partikulären organischen Substanz (POM) von 63–400 µm und 400–2000 µm gab es keine Anzeichen, dass irgendwelche Luzernerückstände in diese

Fraktionen transferiert wurden. Auf der Basis von $\delta^{13}C$-Werten wurde berechnet, dass Pflanzenreste > 2 mm in der Variante 4 14.7% an Luzernerückständen und 85.3% an Rückständen von Maiswurzeln aufwiesen. Dieses bedeutet, dass 54 µg g^{-1} Boden oder 60% mehr Luzerne-C in dieser Variante und als in Variante 2 wiedergewonnen werden konnte. Die Gründe für diese offensichtliche Verzögerung der Zersetzung von Luzernerückständen und den Problemen des methodischen Ansatzes wurden diskutiert.

8.2 Die Beziehungen zwischen bodenbiologischen und anderen Bodeneigenschaften in salinen und alkalischen Ackerböden des pakistanischen Punjabs

Der Interaktionen zwischen bodenphysikalischen, bodenchemischen und bodenbiologischen Eigenschaften wurden in 30 pakistanischen Böden von alkalischen und salinen Ackerstandorten untersucht, die sich sehr stark im Ausmaß der Versalzung und im Boden-pH unterschieden. Die biologischen Bodeneigenschaften wurden differenziert in Indikatoren für mikrobielle Aktivität (Basalatmung), mikrobielle Biomasse (C, N, und P) und Gemeinschaftsstruktur (pilzliches Ergosterol) mit dem Ziel, deren Eignung als Indikatoren für Bodenfruchtbarkeit in alkalischen und salinen Ackerböden einschätzen zu können. Alle Böden waren salzbeeinflusst, hauptsächlich durch Na^+ und HCO_3^- und deren Textur wurde dominiert vom Schluff. Die Mediane der Gehalte an organischem Boden- C, Gesamt-N und Gesamt-P lagen bei 4.5 mg g^{-1} Boden, 0.45 mg g^{-1} Boden, und 0.66 mg g^{-1} Boden. Im Durchschnitt waren nur 1.0% des Gesamt-P extrahierbar mit 0.5 M $NaHCO_3$. Die Mediane im Gehalt an mikrobiellem Biomasse C, Biomasse-N, Biomasse-P und Ergosterol lagen bei 94 µg g^{-1} Boden, 11 µg g^{-1} Boden, 4.6 µg g^{-1} Boden, und 0.16 µg g^{-1} Boden. Die Basalatmung variierte um einen Median von 8.6 µg CO_2-C d^{-1} g^{-1} Boden und wurde als einzige der biologischen Bodeneigenschaften nega-

tiv von einer zunehmender Salinität beeinflusst. Ergosterol hatte eine negative Beziehung mit dem Boden-pH. Der mikrobielle Biomasse-C/P-Quotient übertraf den organischen Boden-C/Gesamt-P-Quotienten nahezu um das Dreifache, aber der organischen Boden-C/Gesamt-N-Quotient und der mikrobielle Biomasse-C/N-Quotient lagen beide auf einem ähnlichen Niveau von ungefähr 9.0. Aus diesem Grund lagen auch die Quotienten mikrobielle Biomasse-C/organischer Boden-C und mikrobielle Biomasse N/Gesamt-N beide auf einem ähnlichen ungefähr 2.2%. Der mikrobielle Biomasse P/Gesamt-P-Quotient lag um das Dreifache niedriger. Salz-bürtiges Na hatte etwas negative Effekte auf den metabolischen Quotienten qCO_2. Das hohe Niveau des metabolischen Quotienten qCO_2 zeigt, dass die Mikroorganismen der Böden generell durch die salinen und alkalischen Bedingungen gestresst werden, was zu einer niedrigen Effizienz in der Substratnutzung führt. Dieses verstärkt die Bedrohung von weiteren Verlusten an Bodenfruchtbarkeit durch die Reduktion der Bodengehalte an organischer Substanz.

8.3 Zersetzung von Kompost und Pflanzenrückständen in pakistanischen Böden entlang eines Gradienten in Salinität

Drei organische Zuschlagstoffe (Kompost, Maisstroh und Erbsenstroh) wurden zu fünf pakistanischen Böden gegeben, die einen Gradienten in Salinität bilden, um folgende zwei Hypothesen zu testen: (1) Eine Zunahme der Salinität bei hohem pH-Wert vermindert proportional die Zersetzungen der zugegebenen organischen Substrate und der daraus resultierenden Netto-Zunahme an mikrobieller Biomasse. (2) Die Auswirkungen von Salinität übertreffen die Unterschiede in der Qualität der organischen Zuschlagstoffe. Obwohl Salinität die Gehalte an mikrobiellen Biomasse-C, Biomasse-N,

Biomasse-P und Ergosterol sinken lässt, wird der klare Gradient entsprechend der Salzkonzentration in den Böden nicht durch die mikrobiellen Bodeneigenschaften widergespiegelt. Dennoch nahmen die Quotienten mikrobieller Biomasse-C/N und Biomasse-C/P kontinuierlich mit zunehmender Salinität ab. In Gegensatz dazu blieb der Quotient Ergosterol/mikrobielle Biomasse-C in den vier Böden über pH > 8.9 konstant, wurde aber nahezu verdoppelt in dem am stärksten salinen, aber am wenigsten alkalischen Boden. Die Zugabe der drei organischen Substrate ließ immer die Gehalte der untersuchten mikrobiellen Indikatoren ansteigen. Die durch die Zuschlagstoffe induzierte Zunahme war besonders ausgeprägt für den Gehalt an mikrobiellem Biomasse-P und spiegelte den Gesamt-P-Gehalt der zugeführten Substrate wider. Die höchsten probenahmetag-spezifischen mittleren Gehalte an mikrobiellem Biomasse-C und Biomasse-N wurden am Tag 0, unmittelbar nach Zugabe der Substrate gemessen. Die organischen Zuschlagstoffe erhöhten die CO_2-Entwicklungsraten von allen fünf Böden in eine boden-spezifischen und zuschlagstoff-spezifischen Art und Weise, aber ohne klare Auswirkungen der Salinität. Das gleiche traf auf die Mengen an Gesamt-C und Gesamt-N zu, die in den beiden Fraktionen der partikulären organischen Substanz (POM) 63 – 400 µm und > 400 µm gefunden wurden. Der niedrigste Prozentsatz von substratbürtigem CO_2-C-und die höchste Widerfindungsrate von POM-C wurde in der Kompost-Variante beobachtet und das Gegenteil in der Erbsenstroh-Variante. Der als POM wiedergewonnene Prozentsatz von N war wesentlich geringer als der Prozentsatz von C in der Kompost- und in der Erbsenstroh-Variante, aber nicht in der Maisstroh-Variante.

8.4 Interaktionen von Kompost und Triple-Superphosphat auf das Wachstum von Mais (*Zea mays*) in einem salinen pakistanischen Boden

Ein Gefäßversuch in einem Gewächshaus wurde mit verschiedenen Kombinationen von Kompost und Triple-Superphosphatzugabe durchgeführt, um die Interaktionen zwischen Pflanzenwachstum, der Bildung von mikrobieller Biomasse und der Zersetzung von Kompost in einem stark salinen pakistanischen Ackerboden im Vergleich zu einem nicht-salinen deutschen Ackerboden zu untersuchen. Der pakistanische Boden hatte einen zweifach kleineren Gehalt an Ergosterol, einen vierfach kleineren Gehalts an mikrobiellem Biomasse C, Biomasse-N und Biomasse-P, aber einen fast 20fach kleineren Gehalt an $NaHCO_3$ extrahierbarem P. Die Zugabe von 1% Kompost hatte immer positive Auswirkungen auf die mikrobiellen Eigenschaften und ebenso auf den Gehalt an $NaHCO_3$ extrahierbarem P. Die Zugabe von Superphosphat allein oder in der Variante +Kompost +P oder +P-angereichertem Kompost induzierten eine starke und ähnliche absolute Zunahme an mikrobiellem Biomasse-P in beiden Böden. Der Ertrag in der Kontrollvariante des deutschen Bodens wurde mehr als 10mal größer als im pakistanischen Boden. In den Zugabevarianten nahm der Ertrag von Mais in beiden Böden in der Reihenfolge +P < +Kompost < +Kompost +P < +P-angereicherter Kompost zu. Der maximale Ertrag in der Variante mit P-angereichertem Kompost war fast doppelt so groß wie im deutschen Boden, aber mehr als achtmal so groß im pakistanischen Boden. Die Menge an Wurzel-C in der Fraktion > 2 mm folgte dem Spross-C mit einem relative stabilen Spross-C/Wurzel-C-Quotienten von 4.5 in beiden Böden. Auf der Basis der Unterschiede in $\delta^{13}C$-Werten konnte gezeigt werden, dass Wurzelmaterial 9% zur Fraktion der partikuläre organische Substanz (POM) < 2 mm in den zwei Varianten ohne Kompostzugabe im deutschen Boden und 11% im pakistanischen Boden betrug. Im deutschen Boden wurden 68% des zugegebenen Kompost-C wieder

gefunden, im pakistanischen Boden 64%. Die auf die mikrobielle Biomasse bezogene CO_2-Entwicklungsrate war im salinen pakistanischen Boden wesentlich größer. Die absolute CO_2 Entwicklungsrate und ebenso Kompostzersetzungsrate zeigte keinen starken Unterschiede zwischen den beiden Böden, im Gegensatz zu den Unterschieden im Maisertrag und in der Zunahme an mikrobieller Biomasse.

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