EFFECTS OF SOIL SALINITY INDUCED UNDER IRRIGATED SUGARCANE IN THE ZIMBABWEAN LOWVELD ON SOIL MICROBIAL ACTIVITY

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Abstract

The effects of irrigation induced salinity on the size, activity and efficiency of the soil microbial community (and therefore on the fertility of the soil) was investigated on a sugarcane estate in the Zimbabwean lowveld. Fields were selected that had a gradient in salinity and sugarcane yield. Sugarcane growth was classified as either dead, poor, satisfactory or good cane growth and soil samples (0-15 cm) were taken from these areas. Adjacent undisturbed veld samples were also taken. Cane growth and yields in the various areas of the fields were measured. Soil salinity and sodicity were quantified by measuring the electrical conductivity (EC) and exchangeable cation content and calculating the sodium adsorption ratio (SAR) of the soils.

There was a negative exponential relationship between increasing measured EC and yields of sugarcane indicating that salinity was causing yield decline. This was also true for the effect of salinity on the microbial biomass C, microbial activity (as measured by FDA hydrolytic activity or arginine ammonification rate), the activity of enzymes involved in C (β-glucosidase), P (phosphatase) and S (arylsulfatase) mineralization and potential nitrogen mineralization (as determined by aerobic incubation). The metabolic quotient, which gives an indication of stress and efficiency of the microbial community, increased considerably with increasing salinity. Thus, increasing salinity resulted in a smaller, more stressed, less efficient microbial community, while the turnover rate and cycling of C, N, P and S also decreased. It was concluded that soil salinity not only causes a decline in sugarcane yield through raising the concentration of soluble salts in soil solution but it also has detrimental effects on microbial activity and on mineralization of soil organic C, N, S and P.

Introduction

Salinity is a common problem in agriculture and is usually induced under irrigation. It has been estimated that globally, approximately 40% of land under irrigation is salt-affected (Moore, 1984; Shannon, 1984). This problem is prevalent in soils under irrigated sugarcane (*Saccharum* sp.), especially in areas of low rainfall and high evaporative demand (Haynes and Hamilton, 1999). Poor irrigation and drainage management are normally the main causes of salinisation and, as the water table rises, salts dissolved in the groundwater reach the soil surface by capillary movement. This is in turn is aggravated if the area has high temperatures which increase the rate of evaporation, leaving salt crystals or crusts on the surface or in the top few centimetres of soil. The accumulated neutral soluble salts impede soil fertility (Sarig and Steinberger, 1994) and plant growth (Zahran, 1989 cited in Zahran, 1997) as the ions hinder water uptake and may also be phytotoxic (Zahran, 1997). Serious reductions in sugarcane yield linked to salinity/sodicity have been reported by a number of workers (Meyer *et al.*, 1996; Haynes and Hamilton, 1999).

Whilst the effects of salinity/sodicity on soil chemical and physical properties are well known (Haynes and Hamilton, 1999) their effects on the soil microbial community and microbiological processes remain relatively unstudied (Oren, 1991, 1999; Szabolcs, 1991). Indeed, several recent comprehensive reviews on the effects of salinity and sodicity on soil properties do not include effects on soil microbial activity (Sumner and Naidu, 1997; Keren, 1999; Levy, 1999). Since organic matter (and therefore microbial biomass size and activity) is typically concentrated in the top few centimetres of soil, (Murphy *et al.*, 1998), changes in the chemistry of the surface soil (such as an increase in soil salinity) could greatly affect soil microbial activity. A reduction in microbial activity would be of particular concern. This is because microbially-mediated processes in soils are central to their ecological function. Important processes include degradation of organic residues, transformations of organic matter, mineralization of nutrients held in organic form (e.g. N, S, P) and formation and stabilization of soil aggregates (Dick, 1992).

On a sugar estate in the Zimbabwean lowveld under furrow irrigation it was observed that induced salinity was considerably more marked in the lower than upper slope position of gently sloping fields. Indeed, depressed yields were obvious in the lower part of the toposequence. As part of a wider study relating induced salinity/sodicity to sugarcane yield decline, the effects of increasing salinity on soil microbial activity were investigated.

Materials and Methods

The study was conducted on a sugarcane estate situated in the Zimbabwean lowveld close to the town of Chiredzi (approximately 31°30' longitude, 21°10' latitude). The area receives between 400 and 600 mm rainfall per annum (mainly during summer months), while the temperature ranges between 9 and 38°C (mean of 24°C). The study sites were on vertic soils which were classified as Bonheim form, Windermere family (Soil Classification Working Group, 1991) or Luvic Phaeozem (FAO). Soils had a

clay content of 25 to 35% and their mineralogy was dominated by smectite and montmorillonite, with some accessory vermiculite and kaolinite present.

The estate is under furrow irrigation and fields are typically about 12 ha in size (without subsurface drainage) with a slope of no more than five per cent. Four fields were chosen in which there was an obvious gradient of salinity from the upper to the lower end of the field. These were identified by apparently unaffected sugarcane growth at the upper end (where irrigation water is applied) and poor growth and/or death at the lower end, and by an obvious accumulation of salts at the soil surface at the lower end. Fields were sampled in July 1999 (just prior to harvest) and crops were all at least in their second ratoon.

Four areas of cane were identified down the gradient of each field representing (i) dead and dying cane, (ii) poor cane growth, (iii) satisfactory cane growth and (iv) good cane growth. A plot 2 rows by 2 metres was marked off at each area and this was later hand harvested in order to obtain data on yield and plant growth parameters. Soil samples (0-15 cm) were taken randomly (both within and between sugarcane rows) in an area of 2 metres radius around the marked plots. Ten samples were taken and then were bulked for each area of each field. Additional soil samples were taken from 0-30 cm, 30-60 cm and 60-90 cm depths in a similar manner and analysed for routine salinity/sodicity assessment.

Soil samples were air-dried and sieved (< 2 mm) and transported to the research laboratory at the University of Natal, Pietermaritzburg, South Africa. Electrical conductivity (EC) and the exchangeable cation contents of Na, Ca and Mg were measured in saturated soil paste extract (Beater, 1962). From these data the sodium adsorption ratio (SAR) was determined. Soil pH was measured in water. Soil organic carbon content was determined by the Walkley-Black method (Walkley, 1947).

Due to logistic and border constraints, it was not possible to transport refrigerated field-moist soil samples from the Zimbabwean lowveld to Pietermaritzburg. Thus, to investigate the effects of salinity on soil microbial activity, it was necessary to use re-wetted samples. Air-dried samples were re-wetted to 70% field capacity and incubated at 20 ^{o}C for 30 days prior to analysis.

Microbial biomass C was determined by the method of Vance *et al.* (1987) based on the difference between C extracted with 0.5M K_2SO_4 from chloroform fumigated and un-fumigated soil samples using a K_c factor of 0.38. Basal respiration was determined by placing 30 g of field-moist soil in 50 ml beakers that were put in 1.5 l air-tight jars containing a vial with 20 ml 0.1M NaOH. The soil was incubated in the jars at 22^{o}C in the dark for 10 days. The CO_2 evolved was determined after 2, 5 and 10 days by titration of the NaOH with 0.2M HCl (Anderson, 1982). Fluorescein diacetate (FDA) hydrolysis rate was measured by incubation of soil with FDA and a buffer for 1 hour using the method described by Swisher and Carroll (1980) as modified by Schnürer and Rosswall (1982). The concentration of fluorescein produced was measured colorimetrically at 490nm. Arginine ammonification rate was determined by colorimetric measurement of NH_4-N after soil samples were incubated with an arginine substrate for 3 hours (Alef and Kleiner, 1995). The activity of several soil enzymes was assayed based on the release and quantitative determination of the product in a reaction mixture, the soil samples being incubated with a suitable substrate and appropriate buffer solution. These enzymes are involved in carbon (glucosidase), phosphorus (acid and alkaline phosphatase) and sulfur (arylsulfatase) mineralization and were performed by the methods described by Tabatabai (1994).

Potential nitrogen mineralization was determined by aerobic incubation. Air-dried soil samples were re-wetted to 70% of field capacity and incubated for at 22 ^{o}C for 10 days. Exchangeable NH_4^+ and NO_3^- were extracted from soil samples using 2M KCl (1:50 soil:extraction ratio) at the beginning and end of the incubation and NH_4^+ and NO_3^- were analysed by steam distillation using MgO and Devarda's alloy and titration with 0.005N H_2SO_4 (Bremner, 1965). Mineralized N was calculated as the difference in exchangeable mineral N before and after incubation.

Results and Discussion

It is evident from Table 1 that sugarcane yield declined dramatically from the high to low ends of the furrow irrigated fields and that there was a concomitant increase in EC and, in most cases, exchangeable Na. Crop yields cannot be directly related to measurements shown in Table 1 since samples were only taken to a depth of 15 cm. For example, at site 3 salinity had not reached high levels in the surface 15 cm (Table 1) but the strongly depressed cane yields suggest that salinity was concentrated in the lower soil layers. The additional soil samples mentioned earlier that were taken to a depth of 90 cm are presently being analysed so that a relationship between salinity/sodicity and crop growth can be established.

The study soils had accumulated appreciable concentrations of soluble salts and Na was a major contributor (Table 1). Sodic soils have an SAR greater than 13 and a pH greater than 8.3 (Gupta and Abrol, 1990) and nine of the 20 samples were in this category. In addition, saline-sodic soils have an EC in saturation paste extracts of more than 400 $mS.m^{-1}$ (Sumner, 1995) and two of the nine soils were in this category.

Data in Table 1 also show that at all four sites sugarcane production resulted in a decrease in soil organic matter content compared with undisturbed veld. Such a decrease is common under sugarcane production (Haynes and Hamilton, 1999) and can be attributed to the small amounts of organic matter returned to the soil (the standing crop prior to harvest is burnt to remove trash and the harvested cane is removed) and to tillage-induced soil organic matter degradation (the soil is intensively cultivated about every five years to form furrows prior to replanting and the interrow space is usually ripped annually).

The marked exponential decline in the size of the microbial biomass with increasing EC (Figure 1) demonstrates the extremely negative influence that increasing salinity had on the size of the soil microbial community. Microbial biomass carbon was also found to be positively (by exponential function) related to soil organic carbon content (r^2=64.4). Such results confirm a pattern found in naturally saline soils where the size of the microbial community is usually negatively correlated with

Table 1. Sugarcane yield and several soil chemical characteristics of the soils of the sites.

Site	Sugar cane growth/Veld	Yield ($Mg.ha^{-1}$)	Organic carbon (%)	pH (H_2O)	EC ($mS.m^{-1}$)	Na ($meq.l^{-1}$)	SAR
1	dead	0.0	2.15	8.17	2480	86.4	22.6
	poor	28.3	2.17	9.16	390	33.4	33.4
	satisfactory	159.2	2.04	8.89	278	23.7	29.3
	good	160.6	2.50	9.17	177	14.9	12.8
	veld	-	2.73	9.14	108	11.2	15.1
2	dead	0.0	0.85	9.61	1795	86.0	66.9
	poor	28.3	1.76	8.50	78	6.3	5.8
	satisfactory	106.7	1.74	8.48	54	3.7	4.0
	good	172.2	2.07	8.47	77	3.6	2.6
	veld	-	2.64	8.5	129	9.5	9.7
3	dead	0.0	0.92	7.57	220	19	22.5
	poor	4.3	1.28	9.44	195	16.2	21.8
	satisfactory	81.7	1.23	9.39	151	13.9	20.7
	good	111.5	1.60	8.32	45	2.6	2.9
	veld	-	2.64	8.25	89	4.9	3.7
4	dead	0.0	1.25	8.93	435	37.1	39.1
	poor	6.2	1.61	9.81	298	26.3	26.3
	satisfactory	71.7	1.82	9.41	195	16.2	22.9
	good	114.7	1.68	8.32	56	2.6	2.1
	veld	-	2.66	8.27	95	4.8	3.6

total soluble salts (Ragab, 1993; García *et al.*, 1994) but positively correlated with organic C contents (Zahran *et al.*, 1992; Ragab, 1993).

Basal respiration (a measure of microbial activity) was not closely related to EC (data not shown) and significant correlations were not recorded. The lack of any correlation is surprising since Laura (1974) showed in a laboratory study that total microbial activity (as measured by CO_2 evolution) was generally depressed as soil salinity increased. The reason for the lack of correlation was explained once the metabolic quotient (CO_2 respired per unit of microbial biomass C) was calculated. It increased exponentially with increasing salinity (Figure 1) demonstrating that the quantity of CO_2 evolved per unit of microbial biomass C increased. The metabolic quotient can be used as an index of adverse environmental conditions (including stress and disturbance) for the soil microflora (Wardle and Ghani, 1995). Thus increasing salinity resulted in a smaller, more stressed microbial community which was less efficient in utilizing C resources than its less stressed counterparts. Similarly, Sarig and Steinberger (1994) found that respiratory quotients increased in soils with fluctuating salinity, while García *et al.* (1994) recorded a negative correlation between the respiratory quotient and EC in the arid soils of south east Spain. The negative relationship between microbial biomass C and the metabolic quotient, as observed here, is common (Sparling,

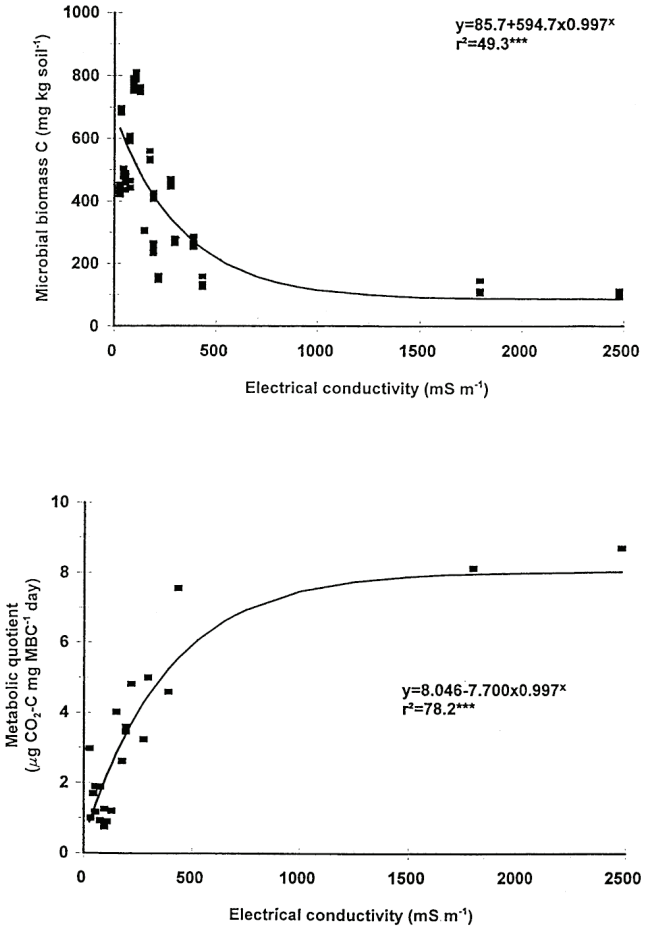

Figure 1. Relationship between electrical conductivity and microbial biomass C and the metabloic quotient for a Zimbabwean Luvic Phaeozem under sugarcane production. Regression equation, line of best fit and statistical significance shown.*P<0.001.**

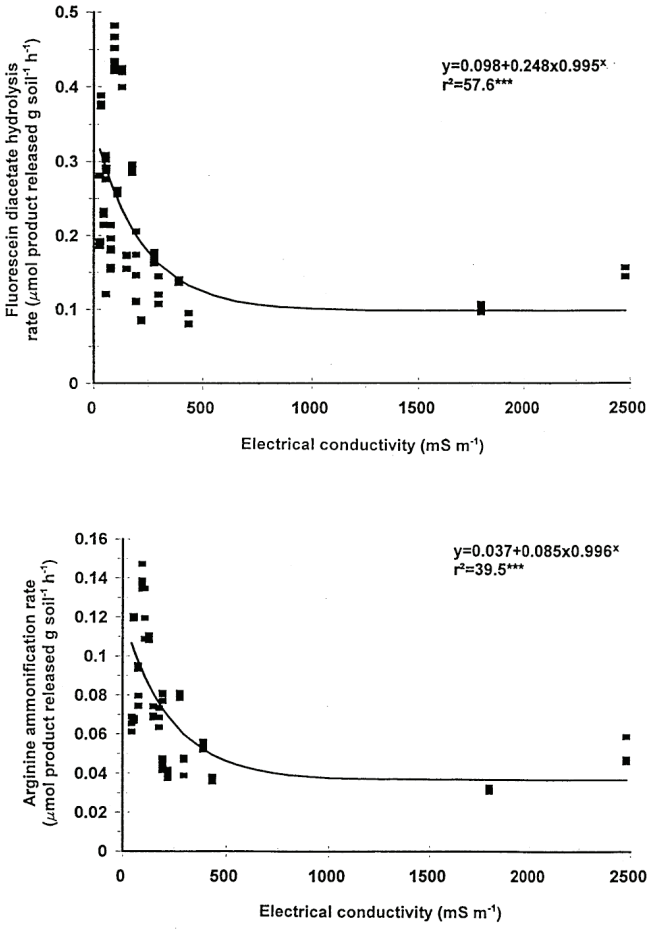

Figure 2. Relationship between electrical conductivity and arginine ammonification rate and fluorescein diacetate hydrolytic activity for a Zimbabwean Luvic Phaeozem under sugarcane production. Regression equation, line of best fit and statistical significance shown.*P<0.001.**

1997) and was noted previously in response to increased salinity by Sarig and Steinberger (1994) in soils in the hot, dry Negev Desert of Israel.

Microbial activity was also estimated by measuring the rate of FDA hydrolysis and arginine ammonification. Arginine ammonification is used as an index of microbial activity since most heterotrophs possess ammonifying capacity and its rate has been found to be closely correlated with microbial activity in laboratory studies (Alef and Kleiner, 1995). The rate of hydrolysis of FDA by soils is considered as an index of overall microbial activity because its hydrolysis is carried out by active cells using an esterase and fluorescein derivatives are hydrolysed by lipases, esterases, and proteases (Schnürer and Rosswall, 1982). Values for both these measurements declined exponentially with increasing EC (Figure 2) demonstrating that not only the size but also the activity of the microbial community was greatly decreased by increasing salinity.

As with microbial biomass C, both FDA hydrolysis and arginine ammonification rates were positively correlated with organic C content (r^2=0.58*** and 0.65*** respectively). Many workers have found that the size and activity of the microbial biomass is positively correlated with soil organic C content (Dick *et al.*, 1988; Haynes, 1999). This is because organic matter is the energy and C source for the bulk of the heterotrophic microbial community.

The reason for the reduced size and activity of the microbial community with increasing salinity is likely to be osmotic stress which is caused by large concentrations of salts in soil solution (Galinski, 1995; Oren, 1999). Osmoregulation becomes a problem and the hypertonic environment tends to dehydrate the microorganisms. Specific ion toxicities (e.g. those of Na and Cl) may also tend to inhibit microbial growth in saline soils (Zahran, 1997).

Although irrigation-induced salinity decreased the size and activity of the soil microbial community, it is evident (Figures 1 and 2) that significant microbial activity persisted under saline soil conditions. Microorganisms that tolerate or require high salt concentrations are termed halotolerant and halophytic respectively. Saline soils generally appear to contain mostly halotolerant microorganisms (Ventosa *et al.*, 1998). Indeed, Zahran (1997) showed that saline soil environments harbour taxonomically diverse microbial groups which exhibit modified physiological and structural characteristics under saline conditions. The majority of halotolerant bacteria can osmoregulate by synthesizing organic osmolytes such as glutamine, proline and glycine but a few of them accumulate inorganic solutes

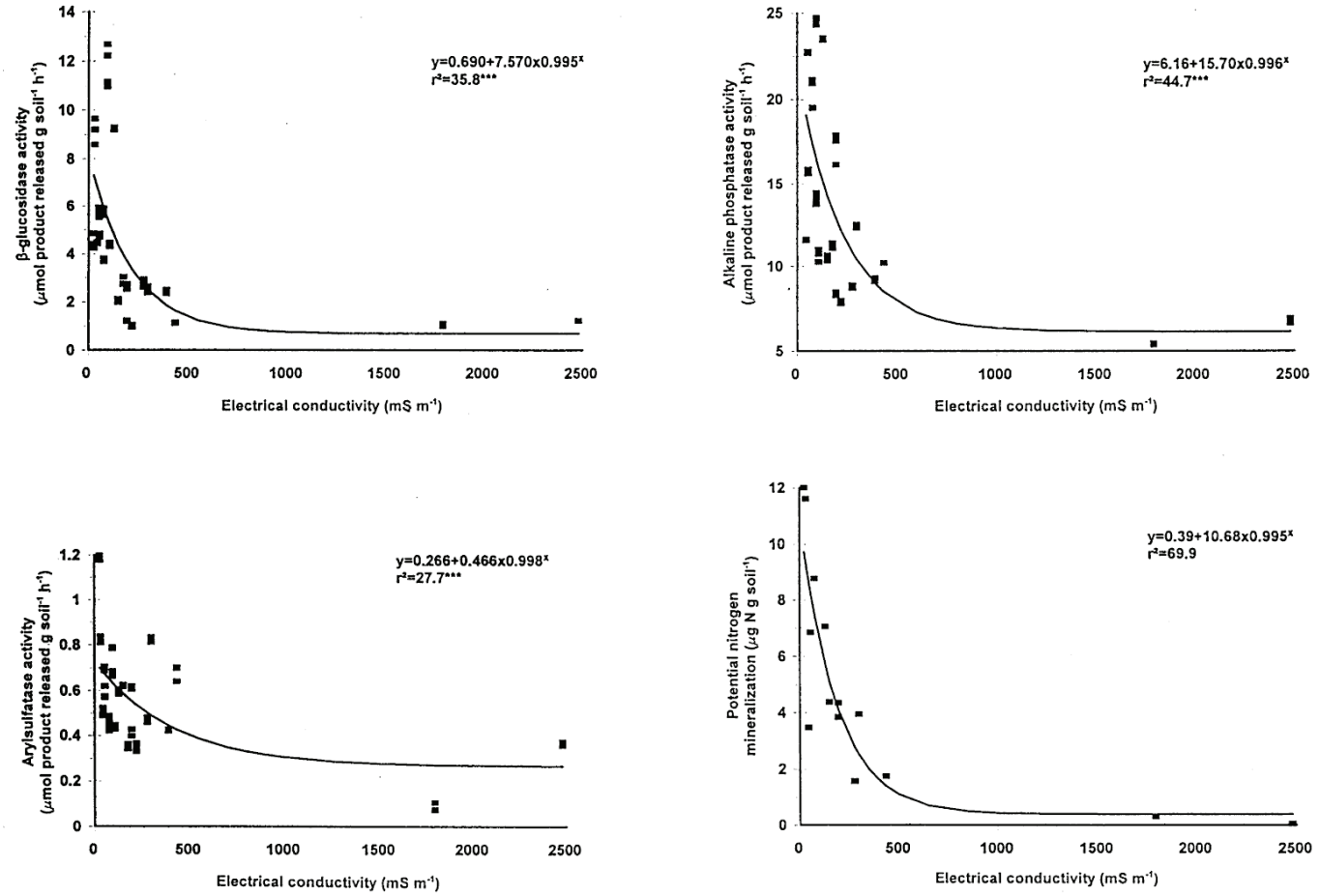

Figure 3. Relationship between electrical conductivity and β-glucosidase activity, alkaline phosphatase activity, arylsulfatase activity and potential nitrogen mineralisation for a Zimbabwean Luvic Phaeozem under sugarcane production. Regression equation, line of best fit and statistical significance shown.*P<0.001.**

(Zahran, 1997; Ventosa *et al.*, 1998). In a recent study, Pankhurst *et al.* (2001) found that agriculture-induced salinity caused a shift towards a less active, less functionally diverse, bacterial-dominated community.

The activity of exo-cellular enzymes involved in C (β-glucosidase), P (alkaline phosphatase) and S (arylsulfatase) mineralization declined exponentially with increasing salinity (Figure 3). The enzyme â-glucosidase catalyses hydrolysis of cellulose into glucose whilst phosphatase and arylsulfatase catalyse the hydrolysis of phosphate and sulfate ester bonds with the release of phosphate and sulfate respectively. The practical implication of these results is that mineralization of soil organic C, P and S is likely to be decreased by salinity. Acid phosphatase activity was also assayed but its activity was not significantly correlated to EC (data not shown). This is possibly related to the high pH of the study soils.

The lowered enzyme activity with increasing salinity will be partially due to a smaller, less active, microbial biomass excreting less enzymes. In addition, high salt concentrations tend to denature proteins through disruption of the tertiary protein structure which is essential for enzymatic activity (Zahran, 1997). In order to counteract such an effect, it is thought that moderately halophytic microorganisms can excrete salt-tolerant enzymes that can carry out their catalytic functions at high salt concentrations (Ventosa *et al.*, 1998).

Although soil microbial activity and C mineralization were decreased by increasing salinity there was no evidence of an accumulation of soil organic matter in the salt-affected soils (Table 1). Indeed, at sites 2, 3 and 4 there was a tendency for a decrease in soil organic C content on the plots where cane was dead. It seems likely that the decreased soil organic matter decomposition was balanced by a decreased input of organic material (particularly in the form of turnover of sugarcane roots) due to the poor cane growth induced by salinity. Where there is no cane there is no significant input of organic material and therefore organic carbon content declines.

The exponential decline in potentially mineralizable N with increasing EC (Figure 3) is in agreement with the findings of other workers (Bandyopadhyay and Bandyopadhyay, 1983). Indeed, although ammonification (the conversion of soil organic N to NH_4^+) can be stimulated by low salt concentrations, it is characteristically inhibited by higher concentrations (McClung and Frankenberger, 1987; Pathak and Rao, 1998). Although Laura (1977), in a laboratory study found that nitrification (conversion of NH_4^+ to NO_3^-) was more sensitive to salinity than ammonification, results presented here have provided no evidence of this. Indeed, the initial mean exchangeable NH_4:NO_3 ratio in soils prior to incubation was 2.34 (range 0.03 to 7.28) and after the 10 day incubation it had decreased to 0.29 (range 0.02 to 0.59) and the ratio was unaffected by increasing salinity (data not shown).

Decreased microbial activity in salt-affected soils, as observed here and by others (such as Mallouhi and Jacquin, 1985; Pankhurst *et al.*, 2001), may have wide implications in the crop-soil system. For example, the microbial community is involved in key soil processes such as formation and stabilization of soil aggregates and decomposition of plant residues as well as mineralization; all these processes will be depressed by increased salinity. Interestingly, both Nelson *et al.* (1996) and Pathak and Rao (1998) noted that decomposition of plant residues in soils was decreased by salinity.

In recent times much research has centred on the use of soil biological properties as indicators of soil health or quality (Pankhurst *et al.*, 1997). The activities of various soil enzymes have been suggested as sensitive, rapid and inexpensive indicators of perturbations to the soil system (Dick, 1992). Our results confirm this. However, of particular interest in this study was the marked increase in metabolic quotient with increased salinity. The metabolic quotient has been shown to be a particularly sensitive indicator of soil pollution (e.g. with heavy metals; Sparling, 1997) and it is evidently also very sensitive to increasing salinity.

Conclusions

This study showed that irrigation induced salinity through a rising water table not only had an extremely adverse effect on plant growth and yield of sugarcane but also had an adverse effect on the size and activity of the soil microbial biomass and on soil microbial processes essential for maintenance of soil quality. An implication of this is that soil fertility will be decreased (particularly due to decreased availability of N, S and possibly P) and this will add to salinity *per se*. as an additional growth limiting factor for crops in salt-affected soils.

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