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Soil pH changes during legume growth and application of plant material

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Abstract During cultivation of legumes soil is acidified due to proton release from roots. As a consequence of proton release, plants accumulate organic anions which may, if returned and decomposed in the soil, neutralize the soil acids. Until now the detailed processes responsible for the change in soil pH after incorporation of plant material have not been completely understood. Using a pot experiment we studied the changes in acid and base in soil during growth of field beans (*Vicia faba* L. cv. Alfred) and after incorporation of the plant material into the soil. Soil pH was significantly decreased by field beans from 6.00 to 5.64 in a cultivation period of 45 days. Proton release amounted to 32.7 mmol H^+ pot^{-1}, which was approximately equivalent to the accumulated alkalinity in the plant shoots (34.4 mmol). Return of field bean shoots caused a significant soil pH increase from 5.64 to 6.29. Within 7 days more than 90% of the added alkalinity was released. After 307 days incubation, soil pH decreased to 5.86 due to nitrification. In a second experiment, maize leaves (*Zea mays* L.), containing various concentrations of nitrogen and at various alkalinities, were incorporated into the soil. Soil pH change was positively correlated to alkalinity and malate concentration and negatively correlated to total nitrogen and water-soluble organic nitrogen of incorporated leaves. It is concluded that the soil acidification caused by legume cultivation can be partly compensated for if crop residues are returned to the soil. Addition of plant material may initially cause an increase in soil pH due to decomposition of organic anions and organic nitrogen. Soil pH may decrease if nitrification is involved. The concentrations of nitrogen and alkalinity of added plant material are decisive factors controlling soil pH change after incorporation of plant material.

Key words Soil pH · Legume · Alkalinity · Decarboxylation · Ammonification · *Vicia faba* L. · Nitrification · *Zea mays* L.

Dedicted to Professor J.C.G. Ottow on the occasion of his 60th birthday

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Introduction

One of the most important factors determining soil fertility is pH, which may, however, be strongly influenced by cultivation of plants and by use of fertilizers. In Australia it was found that soil pH decreased by about one unit as a result of continuous cultivation of clover for more than 30 years (Williams 1980). In a pot experiment with red clover, Mengel and Steffens (1982) showed that soil pH decreased from 7.2 to 4.5 during a vegetation period of 14 months. This decrease in soil pH is mainly attributed to proton release by legume roots (Schubert et al. 1990). It is well known that during proton release by plant roots organic anions such as malate, citrate, and oxalate are accumulated in plants (Bolan et al. 1991; Mengel 1994).

Recently it has been shown that if these organic anions are returned to soil and decomposed by microorganisms soil pH can be increased due to decarboxylation of organic anions (Barekzai and Mengel 1993; Yan et al. 1996). Therefore, one possible way of protecting soil from acidification is by returning the crop residues to the soil. In a field experiment Kretschmar et al. (1991) showed that the application of crop residues of pearl millet for 6 years increased soil pH significantly from 4.54 to 5.69. A similar effect on soil pH after application of plant material was also found by other authors (Hue and Amien 1989; Hafner et al. 1993; Hoyt and Turner 1975; Bessho and Bell 1992). In addition, accumulation of organic anions can be strongly affected by environmental conditions such as supplied nitrogen forms, solution pH, and carbonate concen-

tration of soil solutions (Kirkby and Mengel 1967; Yan et al. 1992; Yang et al. 1994).

Also, there are differences in accumulation of organic anions between plant species. Legumes accumulate higher amounts of organic anions than grasses (Mengel and Steffens 1982). This difference can also influence the effect of application of plant material on soil pH. Hue and Amien (1989) found that the application of leaves of cowpea and leucaena induced a greater increase in soil pH than grass leaves. Similar results were found by Bessho and Bell (1992).

On the other hand, plants contain a large amount of organic nitrogen, such as proteins and amino acids, which can be mineralized to nitrate in soil. Nitrification produces protons and hence acidifies soils. Therefore, the effect of application of plant material on soil pH depends on the composition of the plant material used and the mineralization of organic nitrogen in soil.

The aim of this study was to investigate the effects of the return of crop residues on soil pH and to elucidate the various processes which influence change in soil pH. In addition, an attempt was made to identify important compounds in plant material which determine the effect of application of plant material on soil pH.

Materials and methods

Experiment 1

The purpose of this experiment was to investigate the effects of legume cultivation and the return of plant above-ground material on soil pH.

Soil (soil 1)

The soil used in this experiment was taken from the upper layer of a cultivated loess soil with an original pH of 6.00. Available nutrients of the soil were (kg^{-1} soil) 8.7 mg P, 112 mg K, and 170 mg Mg. The soil was air dried and passed through a 4-mm sieve. The water-holding capacity was 37% (w/w).

Cultivation of legume

Fifteen seeds of field bean (*Vicia faba* L. cv. Alfred) were sown in Mitscherlich pots containing 5 kg soil. To meet the plant demand the soil was fertilized with potassium phosphate (1.5 g KH_2PO_4 pot^{-1}). Eight days later seedlings were thinned to 8 plants pot^{-1}. In total six pots were prepared. Plant cultivation lasted 45 days in the greenhouse and during this time the water content was kept at 70% of water-holding capacity by weighing daily. Plants were harvested at flowering. Plant shoots were cut off and dried at 60 °C for 24 h. After the determination of dry matter, plant shoots were finely ground. At harvest moist soil samples for the measurement of nitrogen and soil pH were collected.

Soil incubation

After harvesting, roots of field bean plants were cut into small segments and mixed with the soil. In three of six pots the finely ground plant shoot material was incorporated into the soil. The amount of applied plant material was exactly the same as the dry matter harvested previously. Plant material was thoroughly mixed with soil and the soil moisture was adjusted to 70% of water-holding capacity. In the other three pots (as controls) no plant shoot material was applied. The pots were covered to avoid water loss by evaporation. Moist soil samples for determination of soil pH and mineral nitrogen were collected during the incubation period.

Experiment 2

The purpose of this experiment was to investigate change in soil pH after incorporation of plant matter with different nitrogen and organic anion concentrations.

Soil (soil 2)

The soil used was taken from the upper layer of a grassland soil developed from loess with an original pH of 6.00. The soil was air dried and passed through a 2-mm sieve. The total carbon content of the soil was 2% and the clay content was 16%. The water-holding capacity was 36%.

Plant material

It is well known that the organic anion concentration of plants may be strongly influenced by the nitrogen form supplied. NO_3^- nutrition results in an accumulation of organic anions, whereas NH_4^+-grown plants show a low organic anion concentration (Kirkby 1968). Therefore, the plant matter used was obtained by growing maize in nutrient solutions with different nitrogen forms and concentrations. Maize seeds (*Zea mays* L. cv. Blissard) were germinated in the dark for 4 days. The seedlings were then transferred into big containers of 50 l nutrient solution. The nutrient solution was composed of (m*M*): 2 $CaCl_2$, 1 K_2SO_4, 1 $MgSO_4$, 0.1 NaH_2PO_4; (μ*M*) 2 $MnSO_4$, 1 H_3BO_3, 1 $CuSO_4$, 0.1 $(NH_4)_6Mo_7O_{24}$. Nitrogen as either $Ca(NO_3)_2$ or $(NH_4)_2SO_4$ was applied at 4 m*M* in the different treatments. Solutions were constantly aerated and changed every 3 days. The first harvest was carried out after a cultivation period of 3 weeks. Half the plants from each treatment were cut off and dried in an oven at 60 °C for 2 days. Dry plant matter was then finely ground. After the first harvest, the nitrogen concentration was reduced from 4 m*M* to 0.4 m*M* in each treatment for further cultivation. The second harvest was conducted 1 week later. The plant material obtained at the second harvest was treated in the same way as at the first harvest. Concentrations of total nitrogen, water-soluble organic nitrogen, NH_4^+, NO_3^-, malate, alkalinity, Ca^{2+}, K^+, Mg^{2+}, and Na^+ of plant materials were analyzed (see below).

Soil incubation

The soil incubation experiment with different plant matters was conducted using small pots (100 ml). Finely ground plant material (1.05 g) was mixed thoroughly with 70 g soil. In this way the content of applied plant matter was adjusted to 1.5 g 100 g^{-1} soil. The water-holding capacity of the soil and plant material mixture was determined. The treatments in this experiment were: control; NH_4^+-grown plant material ($+NH_4^+$); NO_3^--grown plant material ($+NO_3^-$); plant material grown at low NH_4^+ concentration ($-NH_4^+$); and plant material grown at low NO_3^- concentration ($-NO_3^-$). The incubation temperature was kept constant at 25 °C. Water content of the soil was kept at 70% of water-holding capacity. Each treatment was repeated 4 times. Soil samples were taken after various incubation periods and dried at 40 °C for 24 h, then passed through a 1-mm sieve. Soil pH and nitrogen concentration were measured.

Buffer curve of the soil used

The buffer curve of the soil used for the cultivation of field beans in experiment 1 was measured. Ten grams of soil were placed in a small

beaker with a volume of 100 ml. Various amounts of NaOH or HCl solution were added to the soil. The soil moisture was adjusted to 70% of water-holding capacity and protected from evaporation. The incubation was conducted at a constant temperature of 25 °C and lasted for 1 day. Each treatment was repeated 3 times. Soil pH was measured in moist soil samples.

pH measurement

In dry soil

Ten grams of soil were mixed with 25 ml 0.01 *M* $CaCl_2$ and shaken for 30 min. The pH of the clear upper solution was determined 30 min later.

In moist soil

For determination of soil pH 12.20 g moist soil was mixed with 22.8 ml 0.011 *M* $CaCl_2$ solution and shaken for 30 min. The soil pH was measured in the clear upper solution 30 min later.

Measurement of NO_3^-, NH_4^+, and N_{org}

In dry soil

Ten grams soil was shaken in 100 ml 0.01 *M* $CaCl_2$ solution for 2 h. After filtration, concentrations of NH_4^+, NO_3^-, and N_{org} were determined by using an autoanalyzer (Appel and Mengel 1992).

In moist soil

For the determination of nitrogen 25.00 g moist soil was shaken with 195 ml 0.0103 *M* $CaCl_2$ solution for 2 h. After filtration, concentrations of NH_4^+ and NO_3^- in the filtrate were determined by using an autoanalyzer.

Measurement of NO_3^-, NH_4^+, water soluble organic nitrogen ($N_{soluble}$), and malate cocnentrations in plant materials

Dry plant matter (0.1 g) was homogenized in 5 ml deionized water containing 15 mg polyvinylpolypyrrolidone (PVPP) for 10 min and then transferred into a 50-ml volumetric flask. The extraction of NH_4^+, NO_3^-, water-soluble nitrogen, and malate was done in a water bath at 60 °C for 1 h. After filtration, malate was determined using UV test kits from Boehringer (Mannheim, Germany) and NH_4^+, NO_3^-, and soluble organic nitrogen were determined using an autoanalyzer.

Estimation of alkalinity of plant materials

The alkalinity of plant matter was determined according to Jungk (1968); 5 ml/0.1 *M* NaOH solution was added to 0.25 g finely ground plant matter and then dried by heating. The treated plant matter was dry-ashed at 500 °C for 3 h. After cooling, 20 ml 0.1 *M* HCl was added to the ash. The solution was heated to boiling and filtrated. The remaining acid in the solution was titrated by 0.1 *M* NaOH. The alkalinity of plant materials was calculated from the difference in amounts between added NaOH and HCl.

Statistical analysis

Significant differences between treatments were calculated using the *t*-test.

Results

Effect of cultivation with field beans on soil pH (experiment 1)

The cultivation of field beans for 45 days caused a significant decrease in soil pH from the original 6.00 to 5.64 (Table 1). The buffer power of the soil used is shown in Fig. 1. Soil pH changed by 0.55 units for every 1 mmol alkalinity or acid added 100 g^{-1} dry soil. Taking the soil buffer power into consideration, the total amount of protons released by field bean roots during 45 days cultivation was 32.7 mmol H^+ pot^{-1}. Yield of plant shoots (dry matter) was 21.5 g pot^{-1}. Alkalinity of the field bean shoots obtained was 1.60 mmol g^{-1} dry matter. This means 34.4 mmol organic anions pot^{-1} were accumulated in the plant shoots, a value that approximately corresponds to protons released by roots (32.7 mmol H^+ pot^{-1}) during the vegetation period. Total nitrogen concentration of field bean shoots was 3.23%. Dry matter of field bean shoots was 21.5 g pot^{-1}. Therefore, total nitrogen accumulated by field bean shoots was 294 mg N (49.6 mmol N pot^{-1}). Nitrogen in roots was not determined in this experiment.

Effect of incorporation of field bean shoots into soil on pH (experiment 1)

Soil pH increased very rapidly after incorporation of field bean shoots (Fig. 2A). After incubation for 18 h soil pH increased significantly. During the following 7 days soil pH in

Table 1 Soil pH change due to the cultivation of field beans and the return of field bean shoots to the soil. *Values in parentheses* are standard errors [pH_0 original soil pH, pH_1 soil pH after harvest, pH_2 soil pH after return of field bean shoots to soil (18 h after incorporation), pH_3 soil pH at the end of incubation (307 days)]

Treatment	pH_0	pH_1	pH_2	pH_3
+Shoots	6.00	5.64(±0.05)	6.29(±0.02)	5.86(±0.01)
–Shoots	6.00	5.64(±0.05)	5.73(±0.01)	5.84(±0.01)

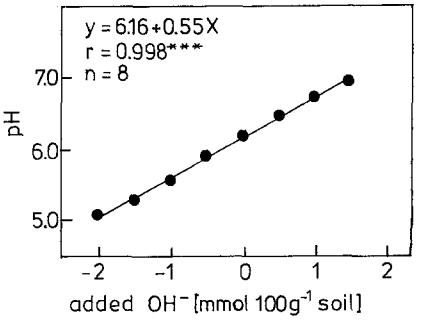

Fig. 1 Buffer power of soil used in experiment 1. Soil was treated with HCl or NaOH and incubated at 25°C, 70% of water-holding capacity for 1 day. Each treatment was repeated 3 times

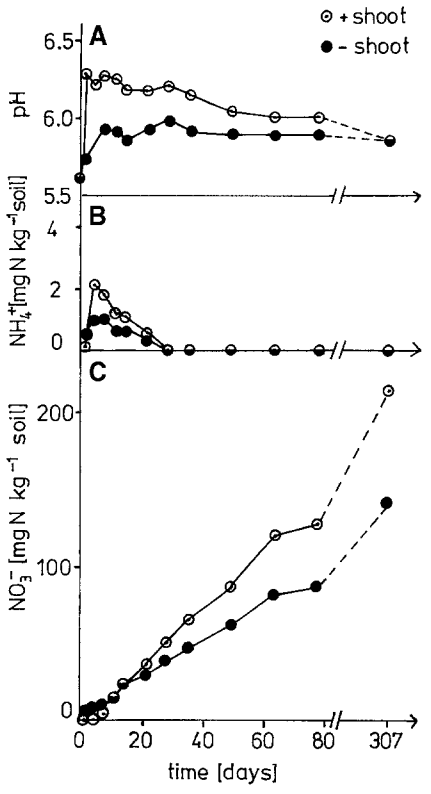

Fig. 2A–C Changes in soil pH, NH_4^+, and NO_3^- concentrations after return of field bean shoots to the soil. Soil incubation was carried out at 70% of water-holding capacity in a greenhouse from June in 1993 to May 1994. Each treatment was repeated 3 times. Standard error of each point was smaller than the size of the symbols. **A** pH, **B** NH_4^+, **C** NO_3^-

the "incorporated field bean shoots" treatment remained relatively constant while the treatment without field bean shoots showed a continuous increase in soil pH. On 7th incubation day soil pH values were 6.28 and 5.94 in the treatments with and without field bean shoots, respectively. Net pH increase due to addition of field bean shoots was 0.34 pH units. Taking the soil buffer power into consideration ($pH = 6.16 + 0.55\ X$), 0.62 mmol OH^- was needed for a soil pH change of 0.34 units 100 g^{-1} soil. This means that in pots with 5 kg soil the alkalinity released from incorporated field bean shoots was 30.9 mmol, corresponding to 90.7% of the total alkalinity supplied with field bean shoots. On the 7th day of incubation the concentration of soil NO_3^- was still low (Fig. 2C), indicating a low rate of nitrification. Up to 80 days of incubation, soil pH in the "without field bean shoots" treatment soil pH remained constant (Fig. 2A). At the end of the incubation (307 days) both treatments showed similar pH levels (Fig. 2A, Table 1), which were lower than the original soil pH.

Soil NO_3^--concentration in the two treatments increased almost linearly with incubation time up to 80 incubation days. The addition of field bean shoots caused a higher rate of increase in soil NO_3^- than the control (Fig. 2C). At the end of incubation (307 days) soil NO_3^- concentration in the "with field bean shoots" treatment was significantly higher than that in the "without field bean shoots" treatment. At this time NH_4^+ concentration of soil in both treatments was so low that it was no longer detectable (Fig. 2B). In total, 366.5 mg N pot^{-1} was mineralized from the incorporated field bean shoots, which amounted to 52.8% of the total nitrogen supplied with the plant matter.

Soil NH_4^+ concentration was fairly low in both treatments during the whole incubation period (Fig. 2B). A slight increase in soil NH_4^+ concentration was found only at the beginning of incubation (4 days), which was higher in the treatment with incorporated plant matter into the soil. From 28 days to the end of incubation soil NH_4^+ concentration was too low to be detected.

Effect of form and concentration of nitrogen supplied on the components of organic and inorganic substances in maize leaves (experiment 2)

The components of organic and inorganic substances of maize leaves were strongly affected by the supplied nitrogen forms (Table 2). The NH_4^+-grown plants accumulated higher amounts of total nitrogen, water-soluble organic nitrogen, and NH_4^+ than NO_3^--grown plants. In contrast, NO_3^--grown plants showed much higher concentrations of malate, NO_3^-, K^+, and Ca^{2+} than NH_4^+-grown plants. Furthermore, alkalinity was higher in NO_3^--grown plants than in NH_4^+-grown plants. Mg^{2+} and Na^+ concentrations of plants showed no significant difference between NH_4^+ and NO_3^--nutrition. With reduced nitrogen concentration in both NH_4^+ and NO_3^- nutrient solutions the plants showed decreased concentrations of total nitrogen and water-soluble organic nitrogen. Concentration of malate and alkalinity of NH_4^+-grown plants were increased with a decrease in NH_4^+ concentration, while concentration of malate and alkalinity of NO_3^--grown plants were decreased with a reduced NO_3^--concentration.

Effect of maize leaf incorporation on soil pH and nitrogen concentration

Incorporation of maize leaves into the soil showed different effects on soil pH and nitrogen concentrations in the

Table 2 Components of organic and inorganic substances in maize leaves cultivated in nutrient solutions with different N forms and concentrations

	4 m*M* NH_4^+	4 m*M* NO_3^-	0.4 m*M* NH_4^+	0.4 m*M* NO_3^-
N_{total}	50.10	36.22	42.71	21.44
$N_{soluble}$ (mg N g^{-1} DM)	20.15	5.50	17.25	3.83
NH_4^+	1.65	0.25	1.58	0.58
NO_3^-	0	6.36	0	1.85
Malate (μmol g^{-1} DM)	5.53	44.73	10.87	38.13
Alkalinity (mmol g^{-1} DM)	0.18	1.28	0.28	0.89
K^+	1.153	2.081	1.234	1.980
Ca^{2+} (mmol g^{-1} DM)	0.061	0.176	0.078	0.147
Na^+	0.045	0.046	0.047	0.042
Mg^{2+}	0.109	0.130	0.110	0.100

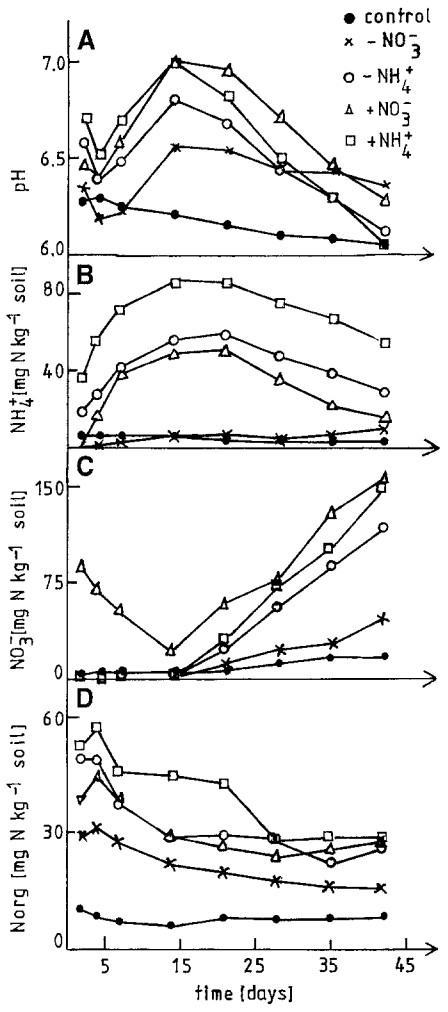

Fig. 3A–D Effects of incorporation of maize leaves on soil pH, $CaCl_2$-extractable NH_4^+, NO_3^-, and organic nitrogen (N_{org}). Amount of incorporated maize leaves was 1.5 g dry matter 100 g^{-1} soil. Incubation was carried out at 25°C, 70% of water-holding capacity. Treatments were: control; incorporation of maize leaves fed with 4 m*M* NH_4^+ concentration ($+NH_4^+$); with 0.4 m*M* NH_4^+ concentration ($-NH_4^+$); with 4 m*M* NO_3^- concentration ($+NO_3^-$); and with 0.4 m*M* NO_3^- concentration ($-NO_3^-$). Each treatment was repeated 4 times. Standard error of each point was smaller than the size of the symbols. **A** pH, **B** NH_4^+, **C** NO_3^-, **D** N_{org}

various treatments. During the first 2 days the soil pH decreased in all treatments supplied with organic matter (Fig. 3A). Then soil pH increased in these treatments until 2 weeks of incubation followed by a pH decrease. At the end of the incubation period soil pH was 6.05, 6.06, 6.11, 6.29, and 6.36 for the control, $+NH_4^+$, $-NH_4^+$, $+NO_3^-$, and $-NO_3^-$ treatments, respectively, and thus higher than the original pH (pH=6.0). pH values obtained at the end of the incubation period were positiviely correlated with the malate concentration (r=0.94) and the alkalinity (r=0.89) in the incorporated plant matter and negatively correlated with the total N concentration (r=–0.94) and soluble N in the plant matter (r=–0.99). The multiple regression for the pH obtained at the end of the incubation period with the alkalinity and the total N concentration in the plant matter as variables yielded a coefficient of determination of R^2=0.997.

Up to 14 days incubation, soil NO_3^- concentration was low in all treatments except for the $+NO_3^-$ treatment, in which NO_3^- concentration was significantly higher than in the other treatments (Fig. 3C). This was attributed to the high NO_3^- concentration in the incorporated maize leaves (Table 2). In addition, during this time the $+NO_3^-$ treatment showed a progressive decrease in soil NO_3^- concentration, indicating a loss or immobilization of mineral nitrogen. During the following incubation period, soil NO_3^- concentration increased continuously because of nitrification.

The concentration of soil N_{org} was significantly enhanced by the addition of maize leaves (Fig. 3D). In addition, the increase was higher in the treatments with NH_4^+-grown leaves than with NO_3^--grown leaves. This difference between NH_4^+-grown leaves and NO_3^--grown leaves was related to the water-soluble nitrogen concentration in the incorporated leaves (Table 2). During the incubation period, soil N_{org} concentration showed a decrease in all treatments, indicating a mineralization of this organic nitrogen fraction. At the end of incubation (42 days), soil N_{org} still showed significantly higher concentrations in all treatments than in the control.

Discussion

The experiment with field beans confirmed earlier findings of various authors, namely that symbiotically grown legumes acidify the soil. According to Hauter and Steffens (1985), this phenomenon is explained by the fact that legumes supplied symbiotically with NH_3 do not take up nitrate. Nitrate, when taken up by plant cells, recycles H^+, pumped out by the plasmalemma ATPase, back into the cytosol with the effect that the pH in the adjacent soil increases. In cases in which plants are not fed with nitrate, soils are acidified due to the activity of the plasmalemma H^+ pump. This H^+ pump (plasmalemma ATPase E.C.3.6.1.35) is the principal ion pump in plant tissues (Serrano 1989; Mengel and Schubert 1985), bringing about a separation of charge and resulting in a pH difference, protons accumulating in the rhizosphere are equivalent to the OH^- primarily produced by the H^+ pump. Hydroxyls remain in the cytosol, where they stimulate the carboxylation of PEP (phosphoenol pyruvate), thus leading to the production of organic anions (Smith and Raven 1979). In most plant species the principal representative of organic anions is malate.

Our observation that the amount of H^+ released by the field bean roots was virtually equivalent to the alkalinity found in the above-ground plant matter of field beans confirms the concept that the pH change in cytosol brought about by the H^+ pump induces the synthesis of organic anions.

Organic anions, not organic acids, yield a pH increase when decomposed in the soil, as was shown by Yan et al. (1996) according to the following equation:

$$R - CO - COO^- + H^+ = R - CHO + CO_2$$

In an analogous way also the alkalinity in plant tissues as determined by the method of Jungk (1968) induces a soil pH increase (Barekzai and Mengel 1993). The alkalinity in plant ashes originates from the diffusible and non-diffusible organic anions, of which the counter cations form oxides and carbonates when ashed. As was found for the maize leaves, malate represents only a few percent of the total alkalinity in plant tissues (Table 2). The soil pH increase found immediately after the incorporation of the plant matter into the soil was approximately equivalent to the alkalinity in the plant matter. The decrease in the soil pH found in the later phase of incubation, especially in the treatment with field bean biomass incorporated into the soil, was due to nitrification. Nitrification produces protons. In the treatment with incorporated field bean biomass more nitrate was formed than in the control treatment because of the higher availability of organic nitrogen in the soil.

The trend of soil pH change found in the treatment with incorporated maize leaves was also brought about by the alkalinity in the plant matter and in a later stage by nitrification (Fig. 3). In this experiment obviously an additional factor influenced soil pH. In the first few days of incubation all treatments which had received organic matter showed a pH decrease. It is assumed that this pH decrease resulted from the formation of organic acids produced from sugars in the glycolytic pathway (Yan et al. 1996). In a later stage these acids were decomposed to H_2O and CO_2. The pH minimum is followed by a steep pH increase in all treatments which had received organic matter. Surprisingly this pH increase was also high in the treatments with low alkalinity (Fig. 3 A) in the incorporated biomass and was already observed at the first pH measurement. However, the concentration of soluble organic nitrogen in this biomass was high (Table 2). According to the finding of Yan et al. (1996), not only organic anions, such as malate or citrate, but also amino acids, such as glycine, caused an increase in soil pH when they were decomposed. Therefore, the pH increase in the treatments with low alkalinity is most likely caused by the decomposition of amino acids. Both decarboxylation and ammonification may be responsible for this pH increase because these two processes occurred in parallel in soils during the decomposition of amino acids (Gonzalez-Prieto et al. 1991). The pH trend of the treatments is roughly reflected by the trend of NH_4^+ concentration in the soil (Fig. 3 B). It is therefore tempting to explain the pH increase in the first phase of incubation by the protonation of the NH_3 formed by ammonification. Such a protonation of NH_3 will only have an alkaline effect if the N present in the biomass, mainly in the form of proteins and amino acids, is not protonated. However, for most amino acids at pH 6 the amino group is protonated whereas the carboxylic group is deprotonated. Ammonification of protonated amino group should not consume protons whereas decarboxylation of deprotonated carboxylic group consumes protons and hence increases soil pH. On the other hand, plants fed with NH_4^+ were rich in soluble nitrogen, of which a remarkable proportion may have consisted of diamino acids because an abundant nutrition with NH_4^+ favors their synthesis (Michael et al. 1965). The second amino group of diamino acids such as asparagine and glutamine has a higher pK value than the α-amino group, and therefore may be present in deprotonated form. Ammonification of this amino group can cause a pH increase. Additionally, it must be kept in mind that the increase in soil pH due to decomposition of organic nitrogen is a short-term effect. With ongoing incubation, soil pH decreased again because of nitrification. In this regard, the amount of soil NH_4^+, as substrate for nitrification, is the determining factor for soil pH change. The higher the NH_4^+ concentration, the lower the soil pH at the end of incubation (Fig. 3 A, B).

Additionally, in the present study we found that the application of organic nitrogen with the biomass of maize leaves clearly increased the so-called "N_{org}" fraction (Fig. 3 D), which is the organic nitrogen extracted by the $CaCl_2$ method of Houba et al. (1986). During the course of incubation the concentration of "N_{org}" decreased in all treatments supplied with biomass because of ammonification followed by nitrification. This relationship found for "N_{org}", NH_4^+, and NO_3^- is in agreement with earlier results of Feng et al. (1990).

From the discussion above, it follows that organic matter incorporation into soils influences soil pH mainly by two processes, the decarboxylation of organic anions increasing soil pH and the mineralization including nitrification of organic nitrogen depressing soil pH. Therefore, the concentration of organic anions and organic nitrogen of incorporated biomass are decisive for soil pH change. Generally, older plant leaves are relatively rich in organic anions and low in organic nitrogen and therefore increase soil pH more than young plant matter, for which the reverse is true. In farming systems dependent on legume production it is therefore important to return crop residues to the fields and to avoid nitrate leaching, not only to conserve nitrogen but also to avoid soil acidification. This is especially important in locations of high rainfall and on soils low in clay content (Bolan et al. 1991; Mengel 1994).

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