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Research article

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Abstract – Extracts of plants such as neem (*Azadirachta indica* A. Juss.) and mata-raton (*Gliricidia sepium* (Jacquin)) are used to control pests. However, certain components of neem, such as azadirachtin, can exert a negative effect on fungi and nitrifying bacteria, and, in turn, can impact the C and N cycles in soil. Nutrient cycling might thus be inhibited and affect the sustainability of an agricultural system in which plant extracts are used to control pests. Here, we investigated the effect of neem extract on microbial activity and N mineralization in soil. We studied the effect of neem and mata-raton leaf extracts on bean growth (*Phaseolus vulgaris* L.), nodule formation by *Rhizobium*, soil CO_2 emissions and soil N dynamics. Four treatments were applied: (1) "neem treatment": extracts of neem leaves, (2) "mata-raton treatment": extracts of mata-raton, (3) "chemical treatment": a chemical insecticide, lambda cyalothrin, and (4) "control": untreated plants. Our results show that in non-amended soil the number of nodules in the neem treatment was 18 for beans cultivated. This nodule number was 2.1 times lower compared with the soil treated with lambda cyalothrin (chemical treatment). In manure-amended soil, the number of nodules was 28 in the neem treatment. This nodule number was 1.6 times lower than in the mata-raton treatment. This indicated that neem extracts inhibited *Rhizobium* in soil and nodule formation in bean. In the manure-amended soil, the emission of CO_2 was 1.9 times lower in the neem-treated soil than in the other treatments. The increase in the concentration of NO_3^- was 1.03 mg N kg^{-1} soil day^{-1} in the neem treatment and 4.1 times lower compared with the other treatments. As such, microbial activity was inhibited by the neem extracts when added to the manure-amended soil. It was found that application of neem leaf extract inhibited microbial activity and reduced nodule formation in bean, but lambda cyalothrin or leaf extracts of *Gliricidia sepium* did not.

aerobic incubation / C and N mineralization / leaf extracts / nodule formation / plant development

1. INTRODUCTION

There is a growing tendency to cultivate crops without using chemical products to control pests (Borad et al., 2001; Kasiwagi et al., 2007). Crops such as coffee or beans, vegetables or fruits grown in such a way have a higher economic value than those cultivated in a more traditional way. More and more farmers in Mexico are now cultivating their land without using chemical products, pesticides and herbicides, so that their crops with a higher economic value can be exported to the USA or the European Community. Pests are difficult to control on organically farmed crops so bio-insecticides, such as bacteria, fungi or botanical pesticides, i.e. plant extracts, are used to control them (Borad et al., 2001; Nathan et al., 2007). Neem, *Azadirachta indica*, A. Juss, of the Meliaceae family, has been investigated intensively for its potential as a botanical pesticide (e.g. Koul et al., 1990; El Shafie and Basedow, 2003). Oil extracts of seeds and water and ethanol extracts of neem leaves are known to inhibit the growth of a great variety of pests (Koul et al., 1990; Amadioha, 2000; Wennergren and Stark, 2000). The bioactivity of neem products has been attributed to the more than 300 components isolated from the different parts of the tree, mostly from the seeds and leaves, which include nimbin, nimbidin and salannin, but the most important of the compounds appears to be the triterpenoid, azadirachtin (Schaaf et al., 2000). These compounds affect the physiology and behavior of a wide range of more than 200 species of phytophagus insects from several orders, bacteria, fungi, viruses, mites and nematodes (Dhar et al., 1998; Coventry and Allan, 2002; Nathan et al., 2007; Kasiwagi et al., 2007).

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However, little is known about how the leaf extracts might affect plant growth and soil processes. Gopal et al. (2007) studied the effect of 10% azadirachtin granules, i.e. alcoholic extract of neem seed kernel mixed with China clay, on the population of bacteria, actinomycetes, fungi, azotobacter and nitrifying bacteria. They found that azadirachtin exerted a negative effect on the microbial communities in the initial 15-day period, but not on *Azotobacter* sp. It has been shown that the production of N_2O through nitrification is affected, i.e. neem extracts can be used as a nitrification inhibitor (Majundar et al., 2000), so other soil processes, such as C and N mineralization or N_2 fixation, might be affected. Additionally, neem is not a native plant of Mexico and *A. indica* originates from India (Koul et al., 1990). Its possible effects on the ecosystems of South Mexico are difficult to foresee, but neem grows abundantly and could easily replace native plant species. Additionally, leaves of neem accumulate under its canopy and it remains unknown how that organic material would affect fauna and flora. Mexico has an extremely rich flora and a lot of plants are used by the local population to control pests. *Gliricidia sepium* (Jacquin), locally called '*mata-raton*', is a leguminous tree and belongs to the family of the Fabaceae. *G. sepium* originates from Central America, but can now be found throughout the world. It is used in many tropical and sub-tropical countries as live fences and sometimes as fodder. It is also used in Mexico to repel insects, so it has potential as a botanical insecticide.

Bean, *Phaseolus vulgaris* L., originating from the Americas, is now cultivated throughout the world and in Mexico, 800 000 ha are cultivated yearly with average yields of 629 kg ha^{-1} and protein contents of 27% (Ortega and Ochoa, 2003). It is often the main or only protein source for poor farmers (Broughton et al., 2003). Apart from its nutritional value, bean is a leguminose and through its symbioses with rhizobia, an N_2 fixator, increases soil nitrogen contents and thus soil fertility. The growth and yields of bean in Chiapas, Mexico, are easily inhibited by soil- or air-borne pests so it is a plant that can be used to investigate the potential of neem and mata-raton as bioinsecticides and their effect on plant growth, nodule formation and soil processes. We investigated how extracts of neem and mata-raton leaves, when applied to soil cultivated with beans, affected plant growth, nodule formation and dynamics of C and N over the growing season. Lambda cyalothrin, Karate or WARRIOR® (Syngenta, Willmington, Del., USA) was used as a chemical control (Farmer et al., 1995). The dynamics of C and N in soil were monitored in an aerobic incubation experiment at the end of the first growing season. The objective of the study was to investigate the effect of leaf extracts of neem, a plant species introduced in Mexico, and mata-raton, an endogenous species, on bean growth, nodule formation and dynamics of C and N in soil.

2. MATERIALS AND METHODS

2.1. Extraction of leaves

Twenty mature neem (*A. indica*) and 20 mata-raton (*G. sepium*) trees were selected at random in the South-East of Tuxtla Gutierrez in the state of Chiapas in the South of Mexico at 16° 46' 24.21" latitude north and 93° 10' 22.48" longitude west (Figs. 1, 2). Its average altitude is 600 m above sea level and it is characterized by a mean annual temperature ranging from 27 to 33 °C with an average annual precipitation of 400 mm without exceeding 600 mm, mainly from May to October. Five hundred g of leaves were sampled from each tree and pooled. As such, ten kg of leaves was collected. One kg of fresh leaves was washed with water, cut into 2-mm^2 squares, added to 3 dm^3 water and left in the dark for 72 h, which was sufficient to extract most of the active components of neem, and filtered (Montes-Molina, 2003). Extraction procedures were kept as simple as possible, as the technique used for extraction should be applicable by the local and often very poor farming community. Lambda cyalothrin, which served as a chemical insecticide, was obtained from Syngenta, Willmington, Del., USA.

Figure 1. Mata-raton (*Gliricidia sepium* Jacquin), approximately three years old.

2.2. Soil sampling

Two different soils were used in the experiment. First, soil was obtained from an agricultural field cultivated with bean in Ocozocoautla de Espinosa, Chiapas, Mexico. Three plots of 400 m^2 were defined and soil was sampled at random by augering the 0–15 cm top layer with a stony soil auger, 7 cm in diameter (Eijkelkamp, Nl). The soil from each plot was pooled,

Figure 2. Neem (*Azadirachta indica* A. Juss.), approximately 15 years old.

and as such three soil samples were obtained. The soil from each plot was passed separately through a five-mm sieve and characterized. The soil, with pH 6.6 and water-holding capacity of 380 g kg^{-1}, had an organic C content of 56.8 g kg^{-1}, an inorganic C content of 2.8 g kg^{-1} and total N content of 8.9 g kg^{-1} soil, and a water content of 78 g kg^{-1}. The particle size distribution of the soil was 50 g clay kg^{-1} (<0.002 mm), 50 g silt kg^{-1} (>0.002 mm and <0.05 mm) and 900 g sand kg^{-1} (>0.05 and < 2 mm).

Second, part of the soil sampled at Ocozocoautla de Espinosa was mixed with composted cow manure in a 1:3 ratio. The cow manure was composted thermophilically with mechanical turning cycles every 15 days for two months. After mixing the soil with composted cow manure, it had a pH 7.0, water-holding capacity of 660 g kg^{-1}, an organic C content of 75.2 g kg^{-1}, an inorganic C content of 4.7 g kg^{-1} and total N content of 16.2 g kg^{-1} soil, and a water content of 76 g kg^{-1}.

2.3. Cultivation of bean in soil treated with insecticide and leaf extracts

The experiment was done twice: once in the unamended agriculture soil and once in the agricultural soil amended with cow manure, and the same experimental design was applied in both cases. Sixteen sub-samples of 10 kg dry soil from each of the three plots were added to plastic bags 30 cm in diameter and of 40 cm depth. Three bean seeds, variety Negro Grijalva (Villar and López, 2003), were planted in each plastic container and placed in a greenhouse at the Instituto Tecnológico de Tuxtla-Gutierrez. The bean cultivar "Negro Grijalva" is characterized by high yields, fast growth, resistance to bean golden yellow mosaic virus and is often cultivated in Chiapas.

Plants were grown under 60% black knitted shade cloth without temperature control. Plants from each treatment were grouped together and spaced at a distance of 70 cm. Each group of plants was separated 1.8 m from the other groups of plants. The grouped plants were arranged in a randomized complete block design.

Seven days after the plantlets emerged two were discarded. Ten ml of the neem leaf extract or the neem treatment, mataraton leaf extract or the mata-raton treatment, or lambda cyalothrin or the chemical treatment were added to the soil surface of 16 bags, while 16 bags remained untreated, considered the control treatment. The amount of lambda cyalothrin added to the soil surface, i.e. 10 mL of a 0.00125 mL L^{-1} solution, was comparable with the recommended amount used to fumigate beans in the field, 250 mL for 60000 plants per ha. The process of applying lambda cyalothrin and the leaf extracts was repeated every seven days until day 49, i.e. a total of seven applications, as recommended for the fumigation of bean with lambda cyalothrin. Every third day, two liters of tap water were added to each container to irrigate the plants. The bean plants were not fertilized during the experiment.

Plant height, stem diameter, time for flowering and the amount of flowers were monitored during plant growth. After 21 days, 28 days and at harvest, four plastic containers were selected at random from each plot and treatment, i.e. a total of 12 plants per treatment, and the plants were removed from the soil, taking care that the roots remained intact. The aboveground plant material was separated from the roots, both were weighed, pods were counted and weighed, and root nodules were counted and weighed. After harvest, four columns from each plot and treatment were selected at random and used for the aerobic incubation.

2.4. Aerobic incubation

Fifteen sub-samples of 10 g soil from each plot and treatment were added to 120-mL glass flasks and adjusted to 40% water-holding capacity by adding distilled water. Three flasks were chosen at random from each treatment and inorganic N was extracted using 40 mL 0.5 M potassium sulfate (K_2SO_4) solution. The samples were shaken for 30 min and filtered through Whatman® No. 42 paper to provide zero-time samples. The remaining flasks were placed in 940-mL glass jars containing a vessel with 10 mL distilled water (H_2O) to avoid desiccation and a vessel with 20 mL 1 M sodium hydroxide (NaOH) to trap the evolved carbon dioxide (CO_2). The jars were sealed and incubated at 22±2 °C for 56 days. An additional three jars without soil, but containing a vessel with 10 mL distilled H_2O and one with 20 mL 1 M NaOH were sealed and served as controls to account for the CO_2 trapped from the atmosphere. After 7, 14, 28 and 56 days, three jars were selected at random from each treatment. The flasks were opened, the vessel with 1 M NaOH removed and stoppered, and the soil was treated as described for zero-time samples. All remaining jars were opened, aired for 10 min to avoid anaerobicity, resealed and further incubated.

2.5. Chemical analyses

Details of the techniques used to analyze pH, total C, inorganic C, total N, soil particle size distribution, CO_2 trapped in NaOH and NH_4^+, and NO_2^- and NO_3^- in the K_2SO_4 soil extracts can be found in Franco-Hernandez et al. (2003).

2.6. Statistical analysis

Cumulative production of CO_2 was regressed on elapsed time using a linear regression model which was forced to pass through the origin but allowed different slopes (production rates) for each treatment. This approach is supported by theoretical considerations that no CO_2 is produced at time zero and a control (flask without soil) accounted for the CO_2 in the atmosphere. Significant differences between treatments for cumulative CO_2 production rates were determined using the SAS software and PROC MIXED (SAS Institute, 1989).

Significant differences for plant and soil characteristics as a result of the different treatments were determined by analysis of variance (ANOVA) and based on the least significant difference using the General Linear Model procedure (PROC GLM, SAS Institute, 1989). This procedure can be used for an analysis of variance (ANOVA) for unbalanced data, i.e. when some data are missing.

The relationships between the different plant characteristics, i.e. number of pods, yield, nodule weight, nodule diameter, number of nodules, plant height and weight, root length and root weight, were visualized by principal component analysis using the orthogonal/varimax rotation to achieve either small or large component loading. Variables were auto-scaled prior to principal component analysis (Sena et al., 2002). The number of components was determined by the Eigenvalue-one criterion (Kaiser, 1960). Moreover, a scree test (Cattell, 1966) was performed to corroborate primer results: only principal components with Eigenvalues > 1 and that explain > 10% of the total variance were retained. Principal component analysis often reveals previously unsuspected associations among variables and thereby allows interpretation that would not be possible otherwise (Johnson and Wichern, 1998). The matrix of four columns (treatments) and eight lines (plant characteristics) was used for principal component analysis. All analyses were performed using the SAS statistical package (SAS Institute, 1989).

The results of the cultivation of the plants in the agricultural soil and soil with added composted cow manure were pooled. As such, the results were the mean of two soils from three different plots, each cultivated with 144 plants.

3. RESULTS AND DISCUSSION

3.1. Soil treatment and its effect on bean growth

Insecticides are normally sprayed on the cultivated plant to control pests and damage to the plants. In this experiment, the botanical insecticides, i.e. extracts of leaves of neem and mata-raton, and the commercial insecticide, were applied to the soil to see how they might affect plant development, nodulation and soil processes. As such, the effect of the treatment on plant development and soil processes could be investigated.

The weight of the aboveground plant and the root length and weight were not significantly different between the treatments for beans cultivated in soil amended with or without manure (Tab. I). Plant height was affected by treatment for beans cultivated in the manure-amended soil, but not in the unamended soil. Plants were 106 cm and 107 cm high when treated with mata-raton and neem and 1.1 times higher compared with the untreated plants, but 1.1 times smaller than when treated with lambda cyalothrin. The application of lambda cyalothrin to soil led to a better aboveground development of the bean plant. Addition of lambda cyalothrin and, to a lesser extent, extracts of neem and mata-raton presumably reduced soil-borne diseases, thereby stimulating plant growth.

The number of nodules was 18 in the neem treatment for beans cultivated in unamended soil and 2.1 times lower compared with the soil treated with lambda cyalothrin (Tab. I). The number of nodules was 28 in the neem treatment for beans cultivated in manure-amended soil and 1.6 times lower than in the mata-raton treatment. It appears that the neem extract had a negative effect on rhizobia and the subsequent nodulation. The antimicrobial activity of neem extracts is well known (Coventry and Allan, 2002) and it has often been reported that neem inhibits microorganisms in soil and their activity. Kiran and Patra (2003) reported that the meliacins, e.g. nimbin and nimbidin, active ingredients that can be found in neem, are responsible for the inhibition of the nitrification process. Gopal et al. (2007) found that extracts of neem seeds inhibited growth of Azotobacter, Actinomycetes and nitrifiers, while Pyo and Oo (2007) reported that it showed high antibacterial activity on seven bacterial species. It is often believed that the use of extracts of plants to control pests will not have a negative effect on the environment or soil fertility only because they are not synthetic. However, it is clear that extracts of neem affect rhizobia and thus soil fertility. Applying extracts of neem indiscriminately without studying their possible negative side-effects should thus be avoided.

The number of pods was 19 for beans cultivated in the mata-raton-treated unamended soil and 1.4 times higher compared with the control and neem-treated soil, while it was 19 and 2.4 times higher when beans were cultivated in the manure-amended soil (Tab. I). The number of seeds was not affected by treatment when beans were cultivated in unamended soil, but it was when cultivated in manure-amended soil. The number of seeds was 2.6 times higher in beans treated with mata-raton than in untreated ones. The reduction in number of nodules due to application of the neem extract had no significant effect on number of pods or seeds. It has often been shown

Table I. Characteristics of bean plants (*Phaseolus vulgaris*) cultivated in an agricultural soil with or without an amendment of cow manure, treated with leaf extract of neem (*Azadirachta indica*) (NEEM treatment) or mata-raton (*Gliricidia sepium*) (MATARATON treatment), or treated with lambda cyalothrin (CHEMICAL treatment) or left untreated for 90 days (CONTROL treatment).

Characteristics	CONTROL	MATARATON	NEEM	CHEMICAL	LSD[a]
Unamended soil					
Plant fresh weight (g)[b]	76 A	83 A	82 A	95 A	24
Root fresh weight (g)[b]	9.4 A	8.1 A	9.9 A	10.5 A	3.8
Length of roots (cm)[b]	26 A	26 A	24 A	27 A	4
Plant height (cm)[b]	117 A	114 A	114 A	122 A	9
Number of nodules[d]	36 AB	28 AB	18 B	38 A	18
Nodule weight (mg)[d]	85 A	124 A	39 A	108 A	111
Diameter of the nodule (cm)[d]	1.3 A	1.2 AB	0.9 B	1.1 AB	0.3
Number of pods[b]	9 B	13 A	9 B	10 AB	3
Number of seeds[b]	8 A	10 A	9 A	7 A	3
Soil amended with manure					
Plant fresh weight (g)[b]	77 A	90 A	84 A	106 A	41
Root fresh weight (g)[b]	6.7 A	7.0 A	9.8 A	12.1 A	6.2
Length of roots (cm)[b]	27 A	29 A	26 A	32 A	9
Plant height (cm)[b]	95 C	106 B	107 B	117 A	8
Number of nodules[d]	42 AB	45 A	28 B	36 AB	16
Nodule weight (mg)[d]	84 AB	89 A	57 B	71 AB	31
Diameter of the nodule (cm)[d]	1.4 A	1.5 A	1.3 A	1.4 A	0.3
Number of pods[b]	8 B	19 A	8 B	11 B	5
Number of seeds[b]	23 C	59 A	49 AB	37 BC	14.9

[a] LSD: Least significant difference ($P < 0.05$),
[b] Value after 90 days of cultivation,
[c] Values with a different letter are significantly different from each other,
[d] Mean of values measured after 30, 60 and 90 days.

Table II. Correlations between characteristics of bean plants (*Phaseolus vulgaris*) cultivated in an agricultural soil with or without an amendment of cow manure, treated with leaf extract of neem (*Azadirachta indica)* (NEEM treatment) or mata-raton (*Gliricidia sepium*) (MATARATON treatment), or treated with lambda cyalothrin (chemical treatment) or left untreated for 90 days (control treatment). Data were pooled among the four treatments and two soils.

Plant characteristics	Plant height	Plant weight	Root length	Root weight	Nodules Number	Nodules Diameter	Nodules Weight	Number of beans
Plant weight	0.167*							
Root length	–0.013	0.340***						
Root weight	0.153	0.599***	0.222*					
Nodules	–0.044	0.163	0.101	0.160*				
Diameter nodules	–0.034	0.168	0.182*	0.076	0.432***			
Nodule weight	–0.002	0.064	–0.023	0.087	0.736***	0.245*		
Number of beans	–0.147	0.247*	0.192*	0.089	0.084	0.196*	–0.005	
Pods	0.180*	0.362***	0.285**	0.203*	0.078	0.048	0.069	0.328***

* $P < 0.05$; ** $P < 0.001$; *** $P < 0.0001$

that the number of nodules has no significant effect on yields of beans. For instance, Fernández-Luqueño (2005) found that bean plants with approximately 300 nodules had similar yields to those with only 100. It can be assumed that the inorganic N available in soil was sufficient to allow a normal growth of the bean plant. However, it can be speculated that in soils with low N mineralization rates bean growth might be inhibited if the amount of nodules is reduced by the application of neem.

Fresh plant weight was strongly significantly correlated with root weight and length and number of pods (Tab. II). The number of nodules was strongly significantly correlated with their average weight and diameter, while the number of pods strongly significantly correlated with the number of seeds. The number of beans was strongly significantly correlated with number of pods.

Loading for parameters obtained after VARIMAX rotation are given in Table III. Principal component analysis was performed using the different plant parameters. The plant characteristics had three significant PCs: only principal components with Eigenvalues > 1 and that explain > 10% of the total variance were retained. A first principal component (PC1) explained 28% of variation and was related to plant growth and yield. PC1 had positive loading from the number of pods and beans, plant and root weight, and root length. A second

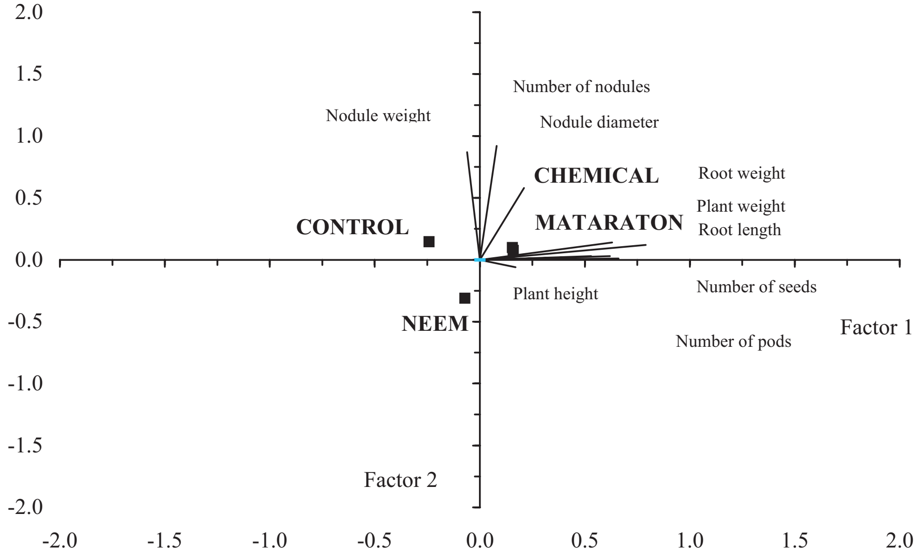

Figure 3. Principal component analysis performed on characteristics of bean, i.e. nodule weight, number of nodules, nodule diameter, root weight, plant weight, root length, number of seeds, number of pods and plant height, cultivated in an agricultural soil with or without an amendment of cow manure, treated with leaf extract of neem (*Azadirachta indica*) (NEEM treatment) or mata-raton (*Gliricidia sepium*) (MATARATON treatment), or treated with lambda cyalothrin (CHEMICAL treatment) or left untreated (CONTROL treatment). The first two factors explained 75% of the variation.

Table III. Rotated loadings on the principal components of bean plant characteristics (*Phaseolus vulgaris*) cultivated in agricultural and cow manure-amended soil, treated with leaf extract of neem (*Azadirachta indica*) (NEEM treatment) or mata-raton (*Gliricidia sepium*) (mata-raton treatment), or treated with lambda cyalothrin (chemical treatment) or left untreated for 90 days (control treatment). Data were pooled among the four treatments and two soils.

Eigenvalues	2.48	1.74	1.24
Proportions	0.28	0.19	0.14
Rotated loading on three retained components[b]			
Plant height	17	–6	74*
Plant weight	79*[c]	12	24
Root length	62	3	–20
Root weight	63*	14	42*
Number of nodules	8	92*	0
Diameter of the nodules	21	58*	–28
Nodule weight	–6	87*	12
Number of beans	53*	3	–59 *
Number of pods	66*	1	–1

[a] Parameters with significant loadings on the within-column principal component,
[b] Only principal components with Eigenvalues > 1,
[c] Characteristics with significant loading.

principal component (PC2) explained another 19% of variation and related to nodule characteristics. PC2 had positive loading from the number of nodules, and their average weight and diameter. A third principal component (PC3) explained 14% of variation and was positively loaded by plant height and root weight and negatively by number of beans. The three principal components explained 61% of variation.

On the scatter plot, the treatments are close to each other (Fig. 3). Plants cultivated in soil treated with lambda cyalothrin and mata-raton can be found in the upper right quadrant. They are close to the X-axis and have a positive value for PC1, indicating heavier plants with longer roots, giving more pods and seeds. Beans cultivated in untreated soil lie in the upper left quadrant. The plants are lighter with shorter roots, i.e. negative PC1 value, but with more, heavier and larger nodules, i.e. positive PC2 value. Beans cultivated in soil treated with neem lie in the lower left quadrant. The nodules of the bean plants have less, lighter and smaller nodules, i.e. negative PC2 value.

Bean plants cultivated in soil treated with lambda cyalothrin and mata-raton developed better than those cultivated in untreated soil or soil treated with neem, while their amounts of nodules were larger. The amount of neem extract that might enter the soil as the consequence of a program to control pests should thus be limited, as plant development and nodule formation is inhibited.

3.2. Production of CO_2 and concentrations of NH_4^+ and NO_3^-

The CO_2 production rate was significantly larger in manure-amended soil than in the unamended soil (Tab. IV). Manure contains large amounts of easily decomposable organic material that increases microbial activity and thus emissions of CO_2. In the unamended soil, the emission of CO_2 was not significantly different between the treated and untreated soil, but it was significantly different between the neem and mata-raton

Table IV. CO_2 production rate (mg C kg^{-1} soil day^{-1}), mean concentration of NH_4^+ (mg N kg^{-1} soil) and rate of increase in NO_3^- concentration (mg N kg^{-1} soil day^{-1}) in agricultural and cow manure-amended soil previously treated with leaf extract of neem (*Azadirachta indica*) (NEEM treatment) or mata-raton (*Gliricidia sepium)* (MATARATON treatment), or treated with lambda cyalothrin (CHEMICAL treatment) or left untreated (CONTROL treatment) aerobically incubated at 22±2 °C for 28 days.

Treatment	CO_2 production rate (mg C kg^{-1} soil day^{-1})	NO_3^- production rate (mg N kg^{-1} soil day^{-1})		NH_4^+ concentration (mg N kg^{-1} soil)
		Unamended soil		
CONTROL	31 AB	0.39 A		3.2 A
MATARATON	23 B	ND		2.1 A
NEEM	37 A	0.39 A		3.8 A
CHEMICAL	28 B	0.15 B		3.4 A
SEE ($P < 0.05$)[b]	4	0.04	MSD ($P < 0.05$)[c]	1.7
		Soil amended with manure		
CONTROL	101 B	5.71 A		5.0 A
MATARATON	110 A	5.67 A		5.3 A
NEEM	53 C	1.03 B		5.7 A
CHEMICAL	109 A	4.19 A		5.1 A
SEE ($P < 0.05$)[b]	3	0.65	MSD ($P < 0.05$)[c]	1.5

[a] Values with a different letter are significantly different from each other ($P < 0.05$),
[b] SEE: Standard error of the estimates ($P < 0.05$),
[c] MSD: Minimum significant difference ($P < 0.05$).

treatments (Tab. IV). In the manure-amended soil, the emission of CO_2 was $\geqslant$1.9 times lower in the neem-treated soil than in the other treatments. The mean concentration of NH_4^+ was similar in the different treatments of the unamended and manure-amended soil, but the increase in the concentration of NO_3^- was 1.03 mg N kg^{-1} soil day^{-1} and $\geqslant$ 4.1 times lower in the neem treatment compared with the other treatments.

In previous experiments, negative effects of lambda cyalothrin on nitrate concentrations, number of nitrifying bacteria and denitrifying bacteria have been reported (Cycon et al., 2006). In the experiment reported here, application of the insecticide lambda cyalothrin had no effect on the C mineralization, but reduced the increases in concentration of NO_3^- in the unamended soil. Applying extracts of leaves of *G. sepium* to the soil surface did not inhibit C and N mineralization, but extracts of neem did in the manure-amended soil. As mentioned before, extracts of neem are known to affect soil bacteria (Mohanty et al., 2008), and this in turn might affect C and N mineralization (Gopal et al., 2007). Inhibition of the N mineralization might reduce the amount of nutrients available to crops, and thus reduce yields.

4. CONCLUSION

Our results show that the number of nodules was lower in bean plants cultivated in soil treated with neem compared with the other treatments. This indicated that neem extracts inhibited *Rhizobium* in soil and/or nodule formation in bean. In the experiment reported here, the emission of CO_2 and the N mineralization rate were also inhibited in neem-treated soil amended with cow manure, but the chemical insecticide and the leaf extracts of *Gliricidia sepium* had no such negative effect. As such, microbial activity was inhibited by the neem extracts. It remains to be seen if the negative effect of the neem extract is temporary or long-lasting. If it is a lasting effect then the use of neem to control bean pests should be discouraged as micro-organisms are affected, which in turn will affect nutrient cycling in soil.

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