Earthworms (Millsonia anomala, Megascolecidae) do not increase rice growth through enhanced nitrogen mineralization

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Abstract

Earthworms have been shown to increase plant growth in 75% of the experiments that have compared plant growth in their presence and absence. However, the relative importance of the different mechanisms advanced to explain such a stimulatory effect has never been tested. In a laboratory experiment we observed increased growth of rice plants in the presence of earthworms (*Millsonia anomala*, Megascolecidae) and demonstrated that enhanced nitrogen release (generally considered as the principal mechanism involved in earthworm positive effect on plants) was not responsible for this result: earthworms had the same stimulatory effect on plant growth (+20 %) irrespective of whether the soil (provided with different amounts of mineral-N fertilizer) was either N-limited or N-saturated. We discuss alternative explanations for the observed variations in rice production

Keywords: Earthworm; Mechanisms affecting plant growth; Mineralization; Nitrogen gradient

1. Introduction

Among the mechanisms by which earthworms modify plant growth at the individual or community levels (Scheu, 2003; Brown et al., 2004), five have been claimed to be responsible for the positive effect noted on plant production: (i) increased mineralization of soil organic matter, which increases nutrient availability (Barois et al., 1987; Knight et al., 1989; James, 1991; Curry and Byrne, 1992; Lavelle et al., 1992; Subler et al., 1997), especially for nitrogen (N), the major limiting nutrient in terrestrial ecosystems; (ii) modification of soil porosity and aggregation (Blanchart et al., 1999; Shipitalo and Le Bayon, 2004), which induces changes in water and oxygen availability for plants (Doube et al., 1997; Allaire-Leung et al., 2000); (iii) production of plant growth regulators via the stimulation of microbial activity (Frankenberger and Arshad, 1995; Muscolo et al., 1998; Nardi et al., 2002; Quaggiotti et al., 2004); (iv) biocontrol of pests and parasites (Stephens et al., 1994; Clapperton et al., 2001; Blouin et al., 2005); (v) stimulation of symbionts (Gange, 1993; Pedersen and Hendriksen, 1993; Furlong et al., 2002).

Enhanced N mineralization is the best documented mechanism and is generally thought to be the most important. However, despite 313 studies on earthworm effects on plant growth, (Brown et al., 1999; Scheu, 2003), no attempts have been made to assess the relative importance of each of these potential mechanisms (Scheu, 2003; Brown et al., 2004). Here, in a particular experimental case, we evaluate the importance of enhanced mineralization by growing rice fertilised with different levels of mineral N. If enhanced N mineralization is the main mechanism involved in the stimulatory effect of earthworms on plant growth, earthworm effect should be most important when the N availability in soil is low since earthworm activities provide the major amounts of mineral N. Under the same hypothesis, the stimulatory effect of earthworm activities should disappear when the availability of mineral N in the soil is high, the N surplus due to earthworms becoming negligible.

2. Materials and Methods

2.1 Experimental units

Young rice seedlings (*Oryza sativa*, cv. Moroberekan) were grown for three months under a 600 µmol photons m^{-2} s^{-1} artificial light source, at 28 °C/day and 24 °C/night temperatures and at 75 % +/- 5 % air moisture. Pots (10 cm in diameter) were filled with 1 kg of a sandy ultisol from Lamto savannah (Ivory Coast). Nitrogen is particularly limiting in the soil of the Lamto savannah : 500 mg kg^{-1} total N (Abbadie and Lensi, 1990; Lensi et al., 1992; Gilot, 1997; Lata et al., 1999), 1.5 to 7.5 mg kg^{-1} NH_4^+ and < 1 mg kg^{-1} NO_3^- (Martin, 1990).

2.2 Earthworms

The original soil fauna was eliminated by sieving (2 mm mesh) and freezing the soil (Blouin et al., 2005). The earthworm *Millsonia anomala*, (Omodeo and Vaillaud, 1967), endemic to this region, is a large mesohumic compacting species (Blanchart et al., 1999), whereas the other species, *Chuniodrilus zielae*, (Omodeo and Vaillaud, 1967) has a somewhat larger geographical distribution and is a thin polyhumic decompacting worm (Blanchart et al., 1999). These two endogeic earthworms feed on soil organic matter; consequently, the soil was not amended with organic matter.

Three immature *M. anomala* (0.85 ± 0.20 g biomass) and three *C. zielae* (0.07 ± 0.02 g) were added in each pot according to the treatments. The population density of *M. anomala* was similar to that of natural populations, whereas that of *C. zielae* was slightly lower (Lavelle, 1978). There were four treatments: *M. anomala* alone (M), *C. zielae* alone (Ch), both species present (MCh), and a control without earthworms (C).

Some earthworms died during the experiment but the mortality of *M. anomala* and *C. zielae* had no significant effect on total (P = 0.24 and 0.14 respectively), aboveground (P = 0.34 and 0.12 respectively) or belowground (P = 0.14 and 0.21 respectively) dry biomasses. The mortality was quite similar in all treatments and not related to the N concentrations.

2.3 Fertilizer

Soil mineral N content was increased by watering daily rice plants with a complete fertilizing solution in which all major mineral nutrients and oligo-elements were kept at constant concentrations except N. Since NH_4^+ is the preferred form of nitrogen taken up by rice (Fried et al., 1965; Sasakawa and Yamamoto, 1978), we modified the Hoagland-Arnon solution by replacing $Ca(NO_3)_2$ with $CaCl_2$ and KNO_3 with $NH_4(SO_4)_2$ and adding silicium which is necessary for many grasses. Therefore, the composition of the fertilizer was KH_2PO_4: 2939 µmol l^{-1}; $CaCl_2$: 2495 µmol l^{-1}; $MgSO_4$:

3950 µmol l^{-1}; Na_2SiO_3: 996 µmol l^{-1}; Fe-EDTA (13 % of Fe): 5 mg l^{-1}; Oligo-elements : H_3BO_3: 55 µmol l^{-1}; $MnSO_4$: 20 µmol l^{-1}; $ZnSO_4$: 0,6 µmol l^{-1}; Na_2MoO_4: 0,4 µmol l^{-1}; $CuSO_4$: 0,6 µmol l^{-1}. NH_4^+ was supplied at five different concentrations (0, 25, 100, 400, 1600 µmol l^{-1}) in each of the four fauna treatments, resulting altogether in 20 treatments; each was replicated 3 times.

2.4 The NH_4^+ gradient

The test of our hypothesis requires a range of N concentrations, from deficient to excess N availability. To ensure N-limitation for plant production in the 0 µmol l^{-1} NH_4^+ treatment, we used a soil that only had 0.05 % total N content (Martin, 1990; Abbadie and Lensi, 1990; Lensi et al., 1992; Gilot, 1997; Lata et al., 1999) and we added all the other macro- and micronutrients at adequate concentrations.

NH_4^+ is known to be limiting to rice seedlings growth below 500 µmol l^{-1} in hydroponical conditions (Wang et al., 1993; Kirk, 2001). In our experiments, the 1600 treatment showed clear evidence of NH_4^+ toxicity : excessive N concentration in plant tissues (fig. 1 a) and deficit in root production (Britto and Kronzucker, 2002) (fig. 1 b). This argues in favour of an alleviation of the N-limitation in the 1600 µmol l-1 treatment.

Fig. 1

2.4 Statistical analysis

Two complementary analyses were conducted on our data set: an ANOVA and an ANCOVA. We checked the homogeneity of variances (Bartlett's test) and normality of the residues (Shapiro-Wilk's test) for total, aboveground and belowground biomasses. Residuals met the conditions of homoscedasticity (P = 0.43, 0.69, 0.20 respectively) and normality (P = 0.80, 0.67, 0.22 respectively). With full ANOVA model, the effects of nitrogen, *M. anomala*, *C. zielae* and the interactions between these factors were tested (Table 1). In this analysis, nitrogen was considered as a discrete factor. To visualize our results more easily, an ANCOVA was conducted considering nitrogen as a continuous variable. The effect of nitrogen was modelled using a polynom whose order is statistically determined. The significant factor(s) of the ANOVA (earthworm species) may affect significantly some of the parameters of the polynom, modifying the regression equation as compared with the control one.

3. Results

As expected, the ANOVA showed that the N gradient had a significant effect on plant dry total and above-ground biomasses (Table 1). Comparisons between means (Tukey's test for multiple comparisons) showed that total and above-ground biomasses were not significantly different from the 0 µmol l^{-1} to the 100 µmol l^{-1} treatments, but increased significantly from the 100 to the 400 µmol l^{-1} treatments (respectively +18 and 19.5 %) and from the 400 to the 1600 µmol l^{-1} treatments (respectively +19 and 31 %), showing that plant growth was N-limited at the lowest

NH_4^+ concentrations. No significant effect of the N gradient was observed on below-ground biomass (Fig. 1c). *M. anomala* had a significant effect on total, above-ground and below-ground biomasses. However, its interaction with the N concentration was not significant (Table 1). The positive effect of *M. anomala* on plant production did not depend on the N concentration. Nor *C. zielae* neither its interactions with the N concentration or *M. anomala* had a significant effect on plant production (Table 1).

Table 1

As *C. zielae* had no significant effect in the ANOVA, we realize the ANCOVA to test the effect of *M. anomala*, independently of the presence of *C. zielae* : control (C) and *C. zielae* treatments, and *M. anomala* and *M. anomala-C. zielae* treatments respectively were pooled together.

Table 2

The relationship between plant total and above-ground biomasses with the N-gradient fitted on a second order polynom ($y = a + bx + cx^2$) (Table 2). Below-ground biomass did not vary significantly along the N-gradient ($y = a$). For total and above-ground biomasses *M. anomala* had a significant positive effect on the parameter a, but not on b or c (Table 2; Fig. 2a and 2b). *M. anomala* also had a significant effect on the parameter a for below-ground biomass (Fig. 2c). Taken together, *M. anomala* had a constant positive effect on plant production along the N-gradient : + 20, 16 and 35 % for the total, above-ground and below-ground biomasses respectively.

Fig. 2

4. Discussion

We postulated that if enhanced N mineralization is an important mechanism involved in the positive effect of earthworms on plant growth, earthworms should have a positive effect in a N-limited environment but not in a N-saturated environment, where the enhanced mineralization of N is negligible. Both an ANOVA, taking into account the presence of *C. zielae*, and an ANCOVA, where the presence of *M. anomala* was considered independently of the presence of *C. zielae*, showed that the effect of *M. anomala* was constant whatever the N concentrations. Thus, we can reject the hypothesis that the main effect of *M. anomala* on plant production was due to increased N mineralization. If *M. anomala* would increase plant production through an enhanced mineralization of other nutrients (such as P, K… etc), this would have produced no effect on plant in a N-limited environment, but an increase in plant growth in situations where plant is no longer limited by N. This was not observed in our experiment (Fig. 2) and we therefore reject this hypothesis.

In contrast to *M. anomala*, *C. zielae* had no significant effect on plant biomass. Only a slight positive effect of *C. zielae* on root biomass had been observed previously (Derouard et al., 1997). In our experiment the soil was probably not compacted enough to allow the decompacting effect of *C. zielae* to influence plant growth significantly.

The increase in plant aboveground biomass simultaneously with the absence of an increase in belowground biomass along the N gradient indicates that the rice plants allocated less resource to the root system as N availability increased (Thornley, 1972; Wilson, 1988; Andrews, 1993). In contrast, the presence of *M. anomala* increased both above- and belowground biomasses. Two separate mechanisms were probably responsible for the differing plant responses to the N gradient and to the presence or *M. anomala* (see below).

Since *M. anomala* effect was constant over the N-gradient, our experiment does not support the widespread belief that enhanced mineralization by earthworms is the main causal mechanism to explain the increased rice growth in presence of *M. anomala*. Nevertheless, this mechanism could be important in other plant-earthworm associations. Moreover, our experiment allows the hypothesis of an enhanced nitrogen mineralization to be excluded at high levels of N, but not at low levels: different mechanisms could in fact be involved at different N concentrations. The mechanism(s) responsible for the positive effect on plant growth at high N concentration cannot be clearly identified from our results. Nevertheless, an improvement of soil water or oxygen availabilities by *M. anomala* can probably be ruled out because soil was maintained at an optimal value of 80 % field capacity by daily watering. Control of specialist parasites or the stimulation of symbionts likely did not occur in our experiment because the soil originated from a non-cultivated savannah with no crop-specific parasites or symbionts, and the original soil fauna had been eliminated. We cannot, however, discard the hypothesis that microbial generalist parasites or symbionts may have been controlled or stimulated by *M. anomala*. This leaves the production of plant growth regulators (Frankenberger and Arshad, 1995; Muscolo et al., 1998; Nardi et al., 2002; Quaggiotti et al., 2004) as the probable explanation of the stimulatory effect of *M. anomala* on rice in our experiment. The possibility and the detailed mechanisms of the control of plant physiology via phytohormones secreted into soils by the bacteria associated with earthworms activities should be studied thoroughly.

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Table 1

Factors affecting total, shoot and root biomasses from a three-way ANOVA

	dl	biomass total			aboveground			belowground		
		F	*P*		F	*P*		F	*P*	
Nitrogen	4	8.5	<0.0001	***	18.3	<0.0001	***	0.8	0.53	
M. anomala	1	9.1	0.004	**	8.4	0.006	**	9.4	0.004	**
C. zielae	1	1,0	0.33		1.1	0.29		0.7	0.39	
Nitrogen * *M. anomala*	4	0.9	0.50		0.9	0.45		0.7	0.63	
Nitrogen * *C. zielae*	4	0.9	0.49		0.4	0.78		1.8	0.16	
M. anomala * *C. zielae*	1	0.2	0.64		0.2	0.64		0.2	0.66	
Nitrogen * *M.a.* * *C.z.*	4	1.3	0.28		1.1	0.37		1.7	0.17	

***: p < 0.001, **: p < 0.01, *: p < 0.1, n = 60

Table 2

Polynomial equations determined with the ANCOVA

model: $y = a + bN + cN^2 + dN^3$ coefficients of	dl	biomass total			aboveground			belowground		
		F	*P*		F	*P*		F	*P*	
N (b)	1	18.0	<0.0001	***	42.0	<0.0001	***	0.2	0.68	
N^2 (c)	1	14.5	0.0004	***	33.1	<0.0001	***	0.2	0.64	
N^3 (d)	1	0.03	0.87		0.98	0.33		1.3	0.25	
M. anomala (a)	4	8.7	0.005	**	8.27	0.006	**	8.5	0.005	**
N * *M. anomala* (b)	4	0.3	0.57		0.26	0.61		0.4	0.53	
N^2 * *M. anomala* (c)	1	0.001	0.98		0.0004	0.99		0.01	0.91	
N^3 * *M. anomala* (d)	1	0.8	0.37		1.19	0.28		0.3	0.58	

***: p < 0.001, **: p < 0.01, *: p < 0.1, n = 60

Fig. 1

Rice responses to the N-gradient according to the presence of earthworms. (a) Percentage of N in plants. (b) Shoot:root ratio of plants. Without lines: control without fauna; Horizontal lines: *M. anomala;* Vertical lines: *C. zielae;* Horizontal and vertical lines: *C. zielae* and *M. anomala*. Means ± s.e., n = 60.

Fig. 2

Response of plant dry biomasses to earthworms along a nitrogen gradient. (a) Total biomass, (b) Shoot biomass, (c) Root biomass. Nitrogen concentration was expressed as log(1+[N]) for more clarity. The polynomial equation of curves was obtained by regression analysis, where the significance of the different coefficients was tested. O: control without fauna; Δ: *C. zielae;* +: *M. anomala;* x: *C. zielae* and *M. anomala*. The different regression lines are represented only for the significant factors; solid line: without *M. anomala*, dashed line: with *M. anomala*. n = 60.