Chapter 5.

Asymbiotic Nitrogen Fixation in Savanna and Eucalypt Plantations

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Introduction

The obvious importance of nitrogen (N) availability for eucalypt growth in a nitrogen-poor environment required determining if biological nitrogen fixation (BNF) was occurring. Microbial activity in eucalypt plantations was expected to be strongly reduced by the well known allelopathic effect of the litter (Souto *et al.* 1995; Bernhard-Reversat 1999).

Simple acetylene reducing activity (ARA) measurements provide a qualitative estimation of the actual soil N_2 fixing activity (Roger and Ladha, 1992). The addition of glucose is generally used to estimate their N_2 fixing potential (expressed when C substrates are not limiting). Indeed ARA in the presence of glucose is also an index of the abundance of free-living N_2-fixing micro-organisms in the soil. O'Connell and Grove (1987) found some ARA in the litter of Australian native eucalypt forests. Although the amount of N_2 fixed extrapolated from these measurements was low, BNF could be a relatively significant source of nitrogen in the nitrogen-poor soil of the eucalypt plantations. Crews *et al.* (2000), also using ARA measurements, reported very low BNF values ranging from 0.05 to 1.25 kg ha^{-1} $year^{-1}$ in the leaf litter of *Metrosideros polymorpha* in Hawaii. The aim of this study was to assess the impact of the different methods of exploitation on the N fertility of the surface of tropical soils planted with eucalypts in Congo. The ARA method (Hardy *et al.* 1968) was used.

Material and Methods

Samples

A first series of composite core samples (4 sub-samples) was collected in the first 5 cm of the profile. Four composite samples were collected in savanna and in each of the age series of eucalypt plots (Ep, Er, Et and Eu). Litter samples were separated from the soil with forceps, except for the savanna samples where no litter was present (Table 5.1).

A second series of 12 litter samples originating from the 19-year-old first rotation crop and coppice plots were used (Et and Eu). Litter alone was sampled because none of the previous soil samples exhibited ARA.

ARA measurements

Acetylene reducing activity (Hardy *et al.* 1968) was estimated on the basis of the acetylene/ethylene pic area ratio, assuming no significant change of the acetylene concentration in the flasks. This method of calculation is valid for a consumption of less than 1% of the acetylene introduced (which was the case in all measurements performed) and has the advantage of being independent of the gas pressure within

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Table 5.1 First series of samples for ARA measurements

Plot	Stand type	Plot age (yr)	Sample harvested	Times	Number of sample
Ep	Seedling	6	Litter	0	1-4
Ep	Seedling	6	Soil	0	1-4
Er	Coppice	13	Litter	1	9-12
Er	Coppice	13	Soil	1	9-12
Eu	Seedling	19	Litter	0	13-16
Eu	Seedling	19	Soil	0	13-16
Et	Coppice	19	Litter	2	17-20
Et	Coppice	19	Soil	2	17-20
Savanna	-	-	Soil	-	5-8

the flask and from eventual leakages. Acetylene and ethylene were measured in a FID Varian chromatograph.

Experimental protocols

First experiment

Soil and litter incubations were performed in 60 ml flasks at 30°C. Soils samples (15 g) and litter samples (5 g) were brought to water retention capacity by the addition of 1.5 ml and 5.8 ml water, respectively. Samples were incubated for one day in open flasks. Then the flasks were closed with a rubber stopper and 10 ml acetylene was injected with a syringe in each flask. As ARA measurements performed daily for five days showed no significant activity, flasks were opened, ventilated to replace the flask atmosphere, 2% glucose was added, then the flasks were closed and 10 ml acetylene was again injected. ARA measurements were performed daily for four days and then after 15 days of incubation.

Second experiment

A second series of measurements was performed on all litter samples, which were first incubated in open flasks for five days, then enriched with 2% glucose before performing daily ARA measurement for four days

Third experiment

A second series of 12 litter samples originating from the 19-year-old first rotation crop and coppice plots was tested. Results showed no ARA both in the absence and in the presence of 2% glucose.

In order to check the potential inhibitory effect of the eucalypt litter on ARA and N_2-fixing micro-organisms we conducted an experiment with a temperate calcareous garden soil having received no agro-chemicals. The soil was treated as previously indicated and received the four combinations of two treatments : 2% glucose : + and - ; eucalypt litter extract : + and -. The eucalypt litter extract was prepared by blending 20 g of litter (second set of litter samples) in 20 ml water, magnetic stirring for 1 hour, followed by filtration. The extract was used straight after its preparation.

Results

In the first experiment (first set of samples), samples tested daily for five days with no glucose added showed no ARA. Six of the 36 samples where glucose was subsequently added, exhibited ARA. All were litter samples. Only data dealing with samples exhibiting significant ARA are presented in Table 5.2.

In the second experiment (first set of samples), only four litter samples collected in two of the plots which had significant ARA in the first experiment, showed ARA ranging from 5 to 75 μ moles g^{-1} h^{-1}. Samples exhibiting significant ARA are presented in Table 5.3.

In the third experiment (second set of samples), none of the 12 litter samples exhibited ARA in the absence or in the presence of 2% glucose. Samples of temperate garden soil without glucose addition had no ARA. Samples of temperate garden soil receiving 2% glucose exhibited ARA values ranging from 198 to 367 μ moles C_2H_4 g^{-1} h^{-1}. The addition of eucalypt litter extract resulted in a significant inhibitory effect (33%). Only samples of the temperate garden exhibiting significant ARA are presented in Table 5.4.

Table 5.2 ARA in litter samples incubated with glucose added: first experiment

Plot and sample	Number of sample	ARA (μ moles g^{-1} h^{-1})
Ep, litter	1	61*
Ep, litter	4	88
Er	11	73
Et	17	55
Et	18	80
Et	19	54

*C_2H_2 production stopped after 50 hours

Table 5.3 ARA in litter samples incubated with glucose added: second experiment

Plot	Number of sample	ARA (μ moles g^{-1} h^{-1})
Ep	4	75
Et	17	20
Et	18	60
Et	20	5

Table 5.4 ARA of temperate garden soil samples with or without addition of eucalypt litter extract

Number of sample	Glucose	Litter extract	ARA (μ moles g^{-1} h^{-1})
4	+	-	367
5	+	-	347
6	+	-	245
11	+	+	198
12	+	+	235
13	+	+	217

Discussion and Conclusions

As no significant ARA was observed with soils, even those enriched with 2% glucose, it is concluded that N_2-fixing activity was absent and there is no significant potential for BNF should an easily usable carbon source become available. No significant ARA was observed with litter incubated without additional source of carbon. Some litter exhibited ARA ranging from 5 to 90 μ moles C_2H_4 g^{-1} soil h^{-1} when enriched with 2% glucose. These values are of the same order of magnitude than those observed by Knowles *et al.* (1973) using a sandy soil enriched with glucose (34 to 51 μ moles C_2H_4 g^{-1} soil h^{-1}). Some other reported values (Granhall 1978) are listed for comparison in Table 5.5.

Among 36 samples studied in the Congolese plantations, only the litter of the 19-year-old coppice (samples 17, 18, 19 and 20) showed significant N_2-fixing activity in the four replicates. In the 7-year-old forest, one litter sample among four exhibited a significant ARA on both measurements. In the 13-year-old coppice, only one sample exhibited ARA on the first measurement. The savanna soil has apparently no actual or potential N_2-fixing activity. Indeed, measurements of ARA in the rhizosphere of various plants in the different ecosystems is needed before drawing any conclusion. However, the third experiment definitely indicates an inhibitory effect on BNF of the hydrosolubles present in the eucalypt litter. The litter of the 19-year-old coppice, which showed most frequently ARA in the above experiments also had the lowest content in soluble phenolic compounds (cf. chapter 3). Results confirm the previously reported inhibitory effect of eucalypts on soil microbial activities. The poor ability of crop residues rich in phenolics to support heterotrophic BNF was also reported by Gibson *et al.* (1988). The occurrence of BNF in Australian forests (O'Connell and Grove, 1987) suggests that soil microflora could be more adapted to eucalypt in their native area. Also free-living N_2-fixers might be more sensitive to the inhibitory effect of eucalypt litter than symbiotic N_2-fixers, as no inhibition of symbiotic BNF was observed in mixed-species stands of *Eucalyptus robusta* with either *Casuarina equisetifolia* or *Leucaena leucocephala* (Parrotta *et al.* 1996).

Table 5.5 Other ARA values from soils (Granhall 1978)

Site	Glucose	ARA (μ moles g^{-1} h^{-1})
Lake sediment	-	0.02-0.3
Rice soils incubated	-	10-70
Temperate soil (Sweden) in pine forest: litter	-	0.5
Temperate soil (Sweden) in pine forest: soil Ao	-	0.08
Temperate soil under various plants	-	0
Temperate soil under various plants	+	1-100

Incubations were made with (+) or without (-) glucose added.