

## Sewage sludge application for spontaneous plant restoration of a New Caledonian Ferralsol

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**Abstract.** Soils from New Caledonia typically present poor nutrient content and large quantities of heavy metals such as nickel, chromium and cobalt, resulting in slow plant development. We evaluated the impact of sewage sludge application (0–30 t dry weight (DW) ha<sup>-1</sup>) on the passive revegetation of a former pine plantation. The spontaneously regenerated plant cover was mainly represented by the dominant *Pinus caribaea*, a shrub (*Sannantha leratii*), and a grass (*Costularia comosa*). The density of pine seedlings was significantly higher in the moderately amended zone (1.2 and 0.2 pines m<sup>-2</sup> for 0.5 and 30 t DW ha<sup>-1</sup> respectively). The same tendency was observed for *S. leratii*, but for *C. comosa*, no net change was observed. With no amendment, after 5 years many species were present, although aboveground biomass (0.3 kg m<sup>-2</sup>) was low, whereas, with sludge amendment, aboveground biomass was high (5 kg m<sup>-2</sup>) but diversity low. Amendment increased pine tree heights from 0.15 to 3.92 m with increased amendment from 0 to 30 t DW ha<sup>-1</sup>. The uptake of nitrogen (N) by pine trees was also improved with sludge supply, as was the uptake of phosphorus (P). Regarding *S. leratii*, N and P levels were highest at the sludge dosage of 2 t DW ha<sup>-1</sup>. Carbon storage in *P. caribaea* biomass increased from 0.40 to 180 kg m<sup>-2</sup> with increased amendment applied. Five years after spreading at the highest amendment levels, available soil P remained enhanced. For heavy metal uptake by pine trees, no significant effect of sewage sludge was observed. The optimal dosage to stimulate biodiversity was 0.5–2 t DW ha<sup>-1</sup> but maximal biomass was reached at 8–30 t DW ha<sup>-1</sup>.

**Additional keywords:** biosolids, forest nutrient availability, mine rehabilitation, revegetation, tropical soils.

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### Introduction

Most soils in New Caledonia are derived from ultramafic rocks that have low nutrient content and high levels of heavy metals (Becquer *et al.* 2001). This results in slow plant growth, meaning that coverage of the soil is not rapid and the soil therefore lacks stabilisation. Faster growing and denser plant cover would address the major issue of soil erosion in New Caledonia.

Sewage sludge from wastewater treatment is an organic resource that may be spread on agricultural land, reducing the need for inorganic fertiliser (Kowaljaw *et al.* 2010). This method is used in crop production and has been recommended since the early 1970s in numerous countries, with appropriate regulations to limit potential hazards for human health and to avoid aquifer pollution and over-fertilisation. In European countries, e.g. in France, the maximum allowable 10-year supply is 30 t dry sludge per ha (JORF 1998; Gavalda *et al.* 2005; Kowaljaw and Mazzarino 2007).

Sewage sludge application improves the physical properties of the soil, raising soil porosity, water infiltration and erosion

resistance (Ojeda *et al.* 2008). It also affects chemical properties, increasing soil organic matter and, subsequently, soil fertility through bacterial mineralisation processes (Petersen *et al.* 2003). Such amendment has resulted in maize, wheat and sunflower yields approaching those reached with inorganic fertilisers (Gavalda *et al.* 2005). In addition to agricultural application, the positive effects on plant growth have helped in restoration of degraded sites. Previous studies have demonstrated that sewage sludge enhances plant re-colonisation of former mine sites (Rate *et al.* 2004), post-fire sites (Larchevêque *et al.* 2008) and industrial sites (Cox and Whelan 2000). Moreover, sewage sludge application in forestry sites led to a significant increase in the production of trees such as *Pinus radiata* (Kimberley *et al.* 2004; Wang *et al.* 2004), *P. halepensis* (Fuentes *et al.* 2007), *Quercus ilex*, *P. pinea* (Larchevêque *et al.* 2006) and *P. sylvestris* (Bramryd 2002; Selivanovskaya and Latypova 2006). This use of sludge, rather than for landfill or incineration, presents all the advantages of recycling nutrients.

The demand for wood is high in New Caledonia, and to reduce wood imports, a forestry program was established in the early 1970s. *Pinus caribaea* was chosen for the plantations because it is native to tropical regions (Central America), is fire- and salt-wind resistant, and grows fast. Recently though, this pine has been considered an invasive species in New Caledonia (Richardson and Rejmánek 2004) and forestry is now oriented towards endemic species such as kauris and araucarias. The literature on inorganic fertilisation of plants is abundant; however, little is known about sludge application or spontaneous germination in a former pine forest. The study was conducted to verify the effect of spreading dehydrated sewage sludge onto a Ferralsol to enhance regeneration of the plant cover. The aims were therefore to examine how the sewage sludge affects (i) the spontaneous germination of seeds in the topsoil, (ii) species diversity, and (iii) the growth of these species and their composition in nutrients, carbon and trace elements. We focussed on nickel (Ni), zinc (Zn) and particularly chromium (Cr), which are naturally rich in this soil, and followed how their levels were affected by the amendment in various plant compartments. In addition, some of the endemic species that germinated have been little studied, so the present work was also an opportunity to obtain information about them.

## Materials and methods

### Biosolids description

The municipal aerobic activated sludge wastewater plant in Koutio, New Caledonia, treats effluent only from domestic sewage (population 30 000). After pre-treatment involving screening and desanding, biological treatment in a basin was followed by settling in a clarification tank. The settled matter was passed through a filter press, giving a fresh non-sanitised sludge, with a dry matter (DM) content of 15%. Organic carbon was measured according to ISO normalisation (NF ISO 10694). The raw sludge, analysed after dissolving with the *aqua regia* method, had high nutrient content (nitrogen (N), phosphorus (P),

**Table 1. Sewage sludge composition and limit values permitted in France for sewage sludge application in agriculture**

	Sludge data	Limit values <sup>A</sup>
Water content (% fresh weight)	15	
<i>Composition on a dry matter basis</i>		
Organic matter (%)	77	
Organic carbon (g 100 g <sup>-1</sup> )	22.1	
N (g 100 g <sup>-1</sup> )	6.26	
P (g 100 g <sup>-1</sup> )	2.46	
Ca (g 100 g <sup>-1</sup> )	1.28	
Mg (g 100 g <sup>-1</sup> )	1.12	
K (g 100 g <sup>-1</sup> )	0.88	
Cu (mg kg <sup>-1</sup> )	137	1000
Zn (mg kg <sup>-1</sup> )	591	3000
Cr (mg kg <sup>-1</sup> )	119	1000
Pb (mg kg <sup>-1</sup> )	20	800
Ni (mg kg <sup>-1</sup> )	78	200
Cd (mg kg <sup>-1</sup> )	0.8	10
Fe (mg kg <sup>-1</sup> )	8031	
Hg (mg kg <sup>-1</sup> )	1	

<sup>A</sup>Since January 1998 (JORF 1998).

calcium (Ca) and magnesium (Mg)) and levels of metal trace elements below the French limits (JORF 1998) for agricultural spreading (Table 1).

### Soil sampling and site description

In 2005, a trial was carried out in a former plantation of *Pinus caribaea* to test the effects of sewage sludge on self-restoration of the plant cover from the soil seed bank. The study site was in the south of New Caledonia (22°10'44.48"S, 166°42'41.69"E; altitude 145 m). Meteorological records since the start of the test give the mean annual rainfall as 1800 mm, annual mean temperature 23°C, and average global radiation 1870 J cm<sup>-2</sup> day<sup>-1</sup> (Bonvallot *et al.* 2013).

In each plot, soil was sampled using an Edelman auger (AMS Inc., American Falls, ID, USA) to a depth of 20 cm. Ten units were randomly taken, placed into a polyethylene bag and vigorously homogenised. The soil samples were then dried in the open air for 72 h and sieved to 2 mm. The red-coloured soil was an Acric Ferralsol according to World Soil Reference Base, naturally poor in organic matter as described in Table 2. The pH of the soil was ~5, and as expected for an acid soil, the cation exchange capacity was very low. The soil fertility was poor with

**Table 2. Main physical and chemical properties of the soil (n = 6) and limit values permitted in France for sewage sludge application**

CEC, Cation exchange capacity; EC, exchangeable cations, measured in ammonium acetate

	Soil data	Limit values <sup>A</sup>
pH <sub>water</sub>	5.22	
pH <sub>KCl</sub>	4.90	
Total C (g kg <sup>-1</sup> soil)	14.5	
Total N (g kg <sup>-1</sup> soil)	1.8	
Total P (g kg <sup>-1</sup> soil)	0.004	
CEC (cmol <sub>c</sub> kg <sup>-1</sup> soil)	4.45	
EC K (cmol <sub>c</sub> kg <sup>-1</sup> soil)	0.03	
EC Mg (cmol <sub>c</sub> kg <sup>-1</sup> soil)	0.05	
EC Ca (cmol <sub>c</sub> kg <sup>-1</sup> soil)	0.14	
EC Na (cmol <sub>c</sub> kg <sup>-1</sup> soil)	0.03	
Particle size distribution		
Sand %	76.3	
Silt %	14.8	
Clay %	8.9	

### Analysed after soil alkaline fusion

Fe <sub>2</sub> O <sub>3</sub> (g 100 g <sup>-1</sup> )	26.2	
Al <sub>2</sub> O <sub>3</sub> (g 100 g <sup>-1</sup> )	12.8	
SiO <sub>2</sub> (g 100 g <sup>-1</sup> )	36.7	
Cr <sub>2</sub> O <sub>3</sub> (g 100 g <sup>-1</sup> )	10.14	
TiO <sub>2</sub> (g 100 g <sup>-1</sup> )	0.76	
Co (g 100 g <sup>-1</sup> )	0.02	
CaO (g 100 g <sup>-1</sup> )	0.6	
K <sub>2</sub> O (g 100 g <sup>-1</sup> )	0.02	
MgO (g 100 g <sup>-1</sup> )	0.02	
Mn (mg kg <sup>-1</sup> soil)	790	
Cu (mg kg <sup>-1</sup> soil)	19	100
Zn (mg kg <sup>-1</sup> soil)	356.5 (76.3 <sup>B</sup> )	300
Cr (mg kg <sup>-1</sup> soil)	68 000	150
Pb (mg kg <sup>-1</sup> soil)	<25	100
Ni (mg kg <sup>-1</sup> soil)	1315.3	50

<sup>A</sup>Since January 1998 (JORF 1998).

<sup>B</sup>*Aqua regia* extraction.

low levels of available P; Olsen's modified Dabin's method (Mathieu and Pieltain 2003) gave  $1.2 \text{ mg kg}^{-1}$ . Total N was analysed by the Kjeldahl method (Bremner 1996). Trace element content was determined in *aqua regia* digest; 3 g soil was mixed with 21 mL HCl and 7 mL  $\text{HNO}_3$  for 12 h, then heated to  $150^\circ\text{C}$  for 2 h, according to ISO normalisation (ISO 11466). Trace metals were present in low amounts, except Cr, Zn and Ni. The Cr, analysed by atomic absorption spectroscopy and then polarography, was not in the highly toxic Cr(VI) form but mainly occurred as Cr(III) (oxides and hydroxides). Both *aqua regia* and alkaline fusion extraction gave the same values, except for Zn, where the *aqua regia* value ( $76.3 \text{ mg kg}^{-1}$ ) was within with regulatory requirements ( $300 \text{ mg kg}^{-1}$ ) whereas alkaline fusion dissolved all of the Zn, resulting in higher levels ( $356 \text{ mg kg}^{-1}$ ), which were therefore above the norm. Soil levels of Cr and Ni were also above the norm for soils considered suitable to receive sludge. These were total soil concentrations and not bioavailable concentrations.

The experimental zone comprised two 6-m-long bands covering a total surface area of 0.1 ha (Fig. 1). Within this area, five main plots were marked out and each was separated into five subplots. Each subplot measured 5 by 5 m and was surrounded with a 1-m buffer zone to allow movement around the subplots and to limit subplot interactions.

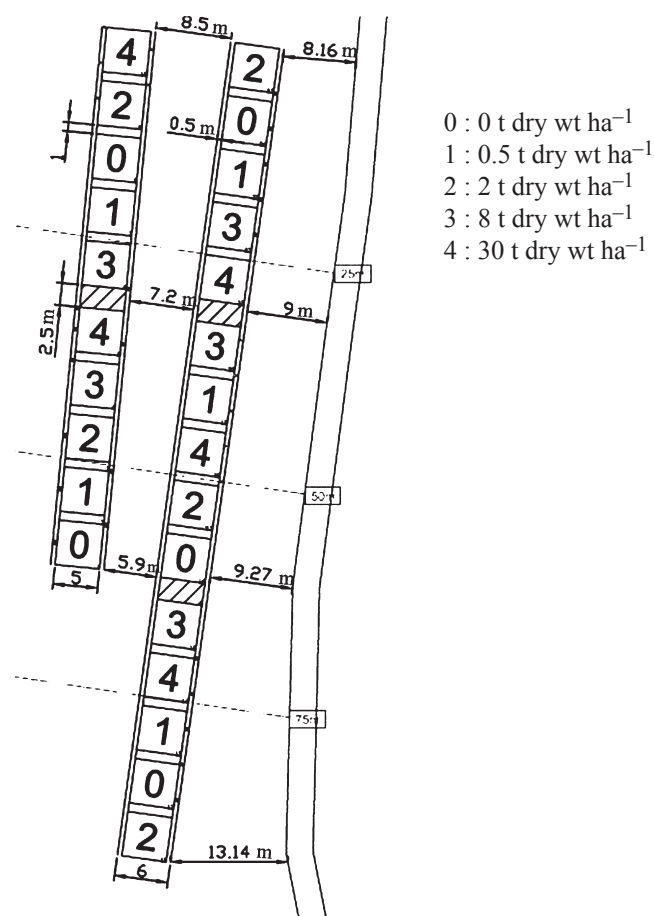


Fig. 1. Experimental site set-up.

### Experimental design

To prepare the experimental plots, both strips of land were completely cleared of vegetation. The stumps of the old planted pines were mechanically removed and the rest of the vegetation was flailed to fragments. The topsoil was tilled to 20 cm depth with a grubber, and the plots were marked out with wooden corner posts.

Sewage sludge was taken at the outlet of the water treatment station and transported directly to the experimental site for immediate spreading in September 2005. All five subplots from each main plot were randomly amended with one of five sludge dosages: 0 (control subplot), 0.5, 2, 8 and 30 t dry weight (DW) ha<sup>-1</sup>. The dewatered fresh sludge was manually spread on the surface of each plot and rotavated into the soil to 20 cm depth.

### Vegetation monitoring and sampling

Observations were conducted on the plants that had emerged from the seeds naturally present (soil seed bank). Species composition was dominated by three plants. Follow-up work focussed on two or three dominant species, based on the data obtained. Measurements were repeated 2.5, 3, 3.5 and 5 years after sludge application. Mortality was calculated between two measurements.

In 2010, the aboveground biomass of all of the dominant species was removed and all other species were sampled on 10% of the surface of each treatment plot. The different parts of the plants (stems, foliage and branches) were separated and weighed. Samples from each treatment and each subplot were dried for 1 week at  $60^\circ\text{C}$  in an oven for analysis. Separation of different plant parts was not the same in all species, i.e. trees were separated into three compartments (foliage, branches and stems) and shrubs into two compartments (stems and foliage). Total aboveground biomass was also measured.

### Chemical analyses

Vegetation samples were digested with concentrated  $\text{HNO}_3/\text{H}_2\text{O}_2$  in a digestion apparatus (DigiPREP: SPS Science, Quebec), and P and heavy metals in the digest were determined using inductively coupled plasma-mass spectroscopy (ICP-MS) (Agilent 7700; Agilent Technologies, Santa Clara, CA, USA).

The mineralisation accuracy was estimated using tomato leaves (1573A, NIST) as a standard reference material. Depending on the metal, recovery was ~80%.

To assess N uptake by plants (except the dominant species), samples were digested in sulfuric acid and hydrogen peroxide at high temperature ( $440^\circ\text{C}$ ) in a digestion apparatus (Digesdahl®, Hach Co., Loveland, CO, USA). Nitrogen was quantified with Nessler's procedure using a DR5000 spectrophotometer (Hach procedure). Carbon and N in the dominant species (*P. caribaea*) were determined by CHN method on powdered samples.

### Statistical analyses

The effect of the amendment on tree heights, mortality, and nutrient and heavy metal concentrations in biomass was evaluated using a one-way analysis of variance (ANOVA). A Kruskal-Wallis one-way ANOVA on the ranks was used

when normality or equal variance tests failed. When the difference between treatments was found to be significant, multiple comparisons were made using Tukey's or Dunn's tests. Comparisons of mean heights of a single plot between two measurement periods were analysed using a *t*-test. All statistical analyses were carried out using SigmaPlot v11 software (Systat Software Inc., San Jose, CA, USA). For trace element comparisons, a two-way ANOVA was carried out to compare treatments, plant parts and their interactions (treatments  $\times$  plant parts).

## Results

### Properties of sewage sludge

Concentrations of N and P were high (6.26% and 2.46%, respectively, in sludge DW; Table 2). Calcium (1.28%) and Mg (1.12%) were also quite high. Therefore, the sludge was relatively rich in the major nutrients (N, P, Ca and Mg). Concentrations of trace elements were low and well below the French limits for land application (JORF 1998), even for Ni and therefore should not limit use through land application.

### Plant cover after 5 years

Five years after spreading of sludge, the plant cover on all subplots of the experimental site was mainly accounted for by the exotic tree *P. caribaea*, a local shrub *Sannantha leratii*, and an endemic grass *Costularia comosa*. As mentioned, *P. caribaea* was introduced into New Caledonia in forestry plantations to enhance wood productivity but is now considered as an invasive species. *Sannantha leratii* (Myrtaceae, previously named *Babingtonia leratii*) is a small shrub (~1.5 m) with rapid growth and low water and sunlight requirements. It is frequently found among the scrub growing in mining spill on ultramafic substrate. *Costularia comosa* (Cyperaceae) grows in clumps of diameter up to 1.5 m. It also contributes to mining scrub.

These three species showed very different behaviour regarding density (Fig. 2). *Pinus caribaea* showed a constant density for the first four treatments (~1.1 trees m<sup>-2</sup>) and then a sharp drop (0.2 trees m<sup>-2</sup>) for the highest dosage treatment. The density of *S. leratii* decreased from 1 to 0.1 shrub m<sup>-2</sup> as the sludge dosage increased. In the case of *C. comosa*, density increased for the first two treatments and then decreased for the last three, higher dosages. The highest density was at 0.5 tDW ha<sup>-1</sup>, while density was zero at 30 tDW ha<sup>-1</sup>.

*Sannantha leratii* and *P. caribaea* were the most recurrent ligneous species in this experiment (Fig. 3), and therefore, they were the only two considered separately for the biomass and nutrient study. *Costularia comosa* was considered only in the 'Other' group because it did not present enough biomass. The 'Other' group included another indigenous grass (*Lepidosperma perteres*), juvenile trees (*Myodocarpus fraxinifolius*, *Alphitonia neocaledonica*, *Eugenia* sp., *Cloezia* sp.), an exotic grass (*Paspalum* sp.), lichen (*Cladonia pycnoclada*) and moss. Litter was defined as all dead plant material deposited on the ground.

The total aboveground biomass is presented (Fig. 3) for the control and four treatment groups (kg dry biomass m<sup>-2</sup>): 0.32 for the control; 1.35 and 1.40 for 0.5 and 2 tDW sludge ha<sup>-1</sup>,

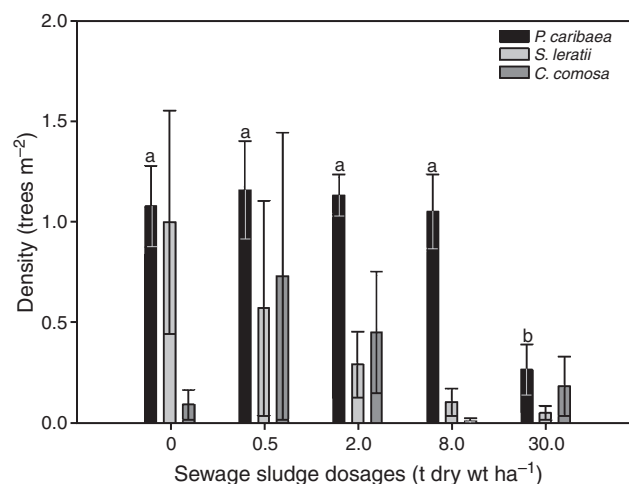


Fig. 2. Density of three dominant ligneous species v. quantity of sludge over the 5 years of amendment. For *P. caribaea*, columns with the same letter are not significantly different at  $P=0.05$  (no differences for the other species because there was too much variability). Capped lines are  $\pm$  s.e.

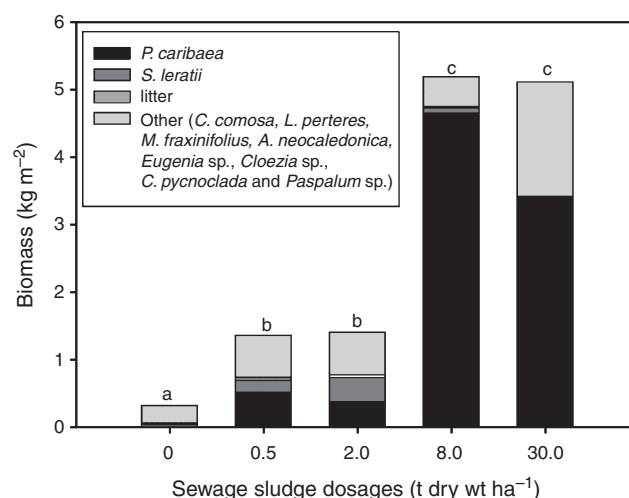


Fig. 3. Total aboveground biomass at 5 years as a function of the dosage of sludge amendment. Columns with the same letter are not significantly different at  $P=0.05$ .

respectively; and 5.17 and 5.10 for 8 and 30 tDW sludge ha<sup>-1</sup>. For the three lowest sludge treatments, *P. caribaea* biomass was less than that of the rest of the biomass (*S. leratii* and 'Other'). For the two highest levels of sludge application, the *P. caribaea* biomass was dominant. For the 30 tDW ha<sup>-1</sup> treatment, 'Other' was mainly represented by *Paspalum* sp. (95%). For *P. caribaea* biomass, the foliage represented 30% of total biomass, stems 60% and branches 10%.

Plant cover was diversified but dominated by pine trees, which alone were to be monitored for the whole 5 years.

### Pine tree mortality and growth

Pine germination was highest on the control plot (Fig. 4). After a 2.5-year growth period, the tree density on each plot decreased from  $1.2 \pm 0.1$  to  $0.3 \pm 0.1$  plants m<sup>-2</sup> with increasing sludge



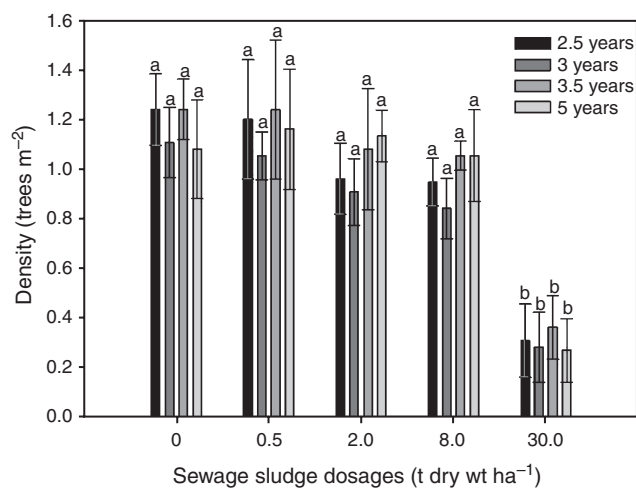


Fig. 4. Pine tree density v. sludge application dosage for the different periods of time. Columns with the same letter are not significantly different at  $P=0.05$ . Capped lines are  $\pm$  s.e.

dosage. Nevertheless, only the plots treated with 30 t DW ha<sup>-1</sup> of sewage sludge presented a significantly lower density of trees during the whole 5 years of monitoring ( $P<0.01$ ).

Between the first and second measurement, the control series presented the highest plant mortality (25%) compared with the highest dose (14%). Between the second and the third measurements, new pine trees emerged so no overall mortality was observed. However, between the third and the last measurement, mortality occurred once more. In general, due to the appearance of new trees, between the first and the last measurement, mortality appeared only for the control plot (20%).

Increasing the sludge level in the soils resulted in an increase in pine height (Fig. 5). Comparison of all five treatments, at each measurement time, indicated a first group with the highest growth (including the treatments 8 and 30 t DW ha<sup>-1</sup>) and a second group with the lowest growth (including the treatments 0.5 and 2 t DW ha<sup>-1</sup>), with no significant differences observed within groups ( $P=0.132$  and  $P=0.051$  for the first and second groups, respectively, after 5 years). Sapling heights in these two groups were significantly greater than in the control group ( $P<0.001$ ). This means that growth was greatest for the highest level of amendment, 30 t DW ha<sup>-1</sup>.

During the five years of the experiment, pine growth rates were linear and can be represented by the slope of each regression. All linear models were significant ( $P<0.01$ ) except in the case of the control plot ( $P=0.056$ ). Moreover, all slopes were significantly different from zero except that for the control plot. With no amendment, the growth rate was 0.018 m year<sup>-1</sup>. For the treatment 2 t DW ha<sup>-1</sup> the growth rate was  $0.20 \pm 0.01$  m year<sup>-1</sup>, for 8 t DW ha<sup>-1</sup> growth rate was  $0.78 \pm 0.05$  m year<sup>-1</sup>, and for 30 t DW ha<sup>-1</sup> growth rate was  $1.10 \pm 0.08$  m year<sup>-1</sup>. Treatments with low dosages showed similar growth rates (0.16 and 0.20 m year<sup>-1</sup> for 0.5 and 2 t DW ha<sup>-1</sup>, respectively;  $P>0.05$ ). For the highest level of amendment, growth rates were significantly higher ( $P<0.001$ ). The growth of trees in the current study (Fig. 5) for treatments 8

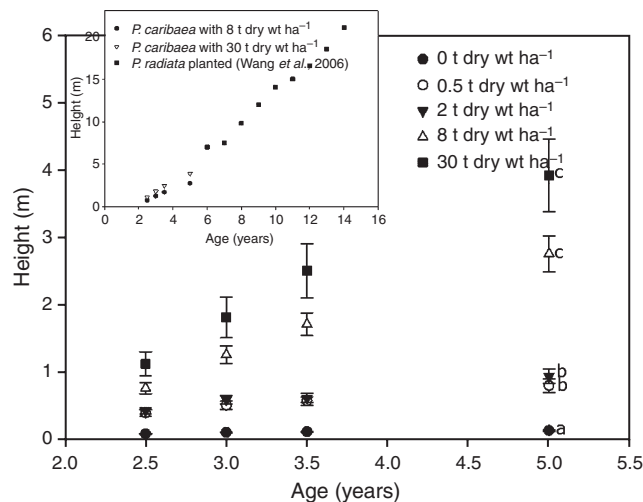


Fig. 5. Pine tree height v. age as a function of the different dosages of amendment in the current study and from Wang *et al.* (2006). Symbols with the same letter are not significantly different at  $P=0.05$ . Capped lines are  $\pm$  s.e.

and 30 t DW ha<sup>-1</sup> agree with the study of Wang *et al.* (2006), who reported similar growth for a plantation of *P. radiata*.

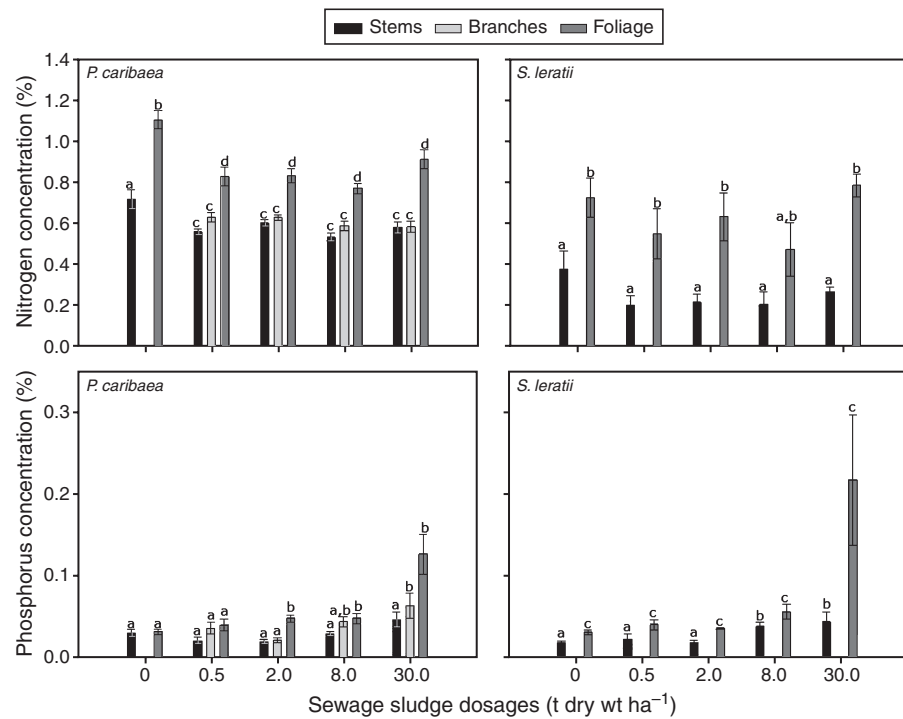
Moreover, application of amendment significantly increased diameter of *P. caribaea* trees and, consequently, tree volumes. There was a direct relationship between height and diameter of pines; after 5 years without sludge, average diameters were 0.01 m for 0.08 m height. With sludge treatment of 30 t DW ha<sup>-1</sup>, average diameters were ~0.09 m for a height of 6 m.

#### Nutrient content in biomass

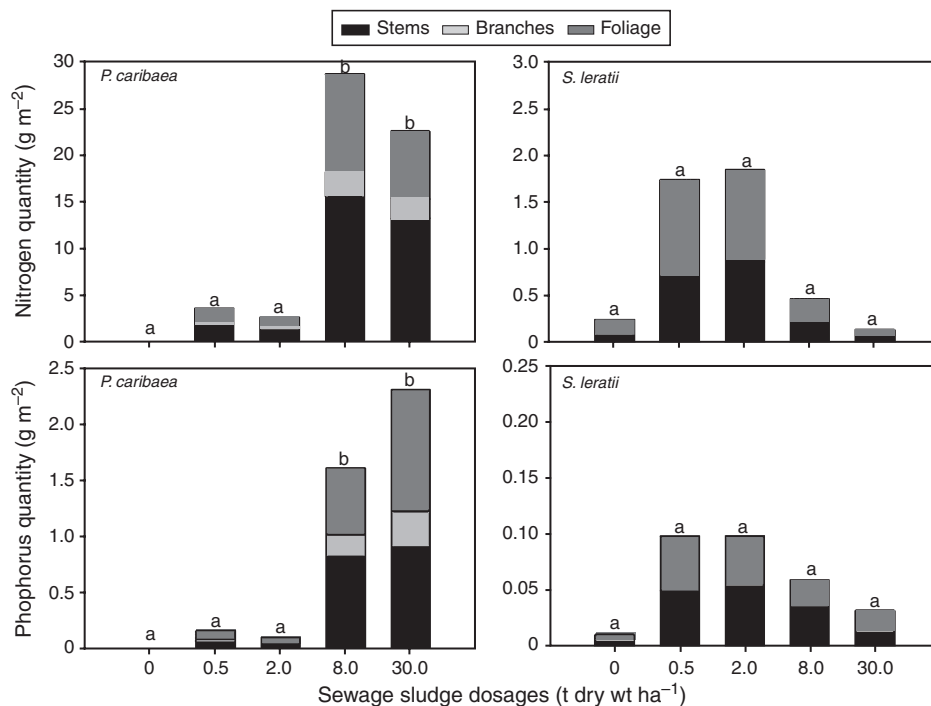
Regardless of species, the distributions of N and P concentrations in the plant were similar (Fig. 6). Across treatments, the concentration of N in *P. caribaea* (Fig. 6) was significantly higher in foliage than in stems and branches ( $P=0.010$  and  $P=0.020$  for stems and branches, respectively). Nevertheless, groupings were not clearly established between treatments for foliage, except for the control plot where foliage N was significantly higher than for all other treatments. The level of N in *S. leratii* (foliage and stems) showed no difference between treatments. However, concentrations were higher in foliage than in stems for each treatment, as for *P. caribaea*.

For P concentrations in *P. caribaea*, no significant differences occurred across treatments except at 2 and 30 t DW ha<sup>-1</sup>, where P in foliage was found to be higher than in branches. Unlike the N concentration in *S. leratii*, the P concentrations were statistically higher (0.22% for foliage and 0.04% for stems) at the highest application levels. Unlike N, in both parts of the plant, P concentration was highest in the higher treatment plots. Whatever the species, there were no statistical differences in the concentrations of P and N.

The biomasses of species were, however, very different, so the quantities obtained for each species were different, by a factor of 10 for *P. caribaea*. Expressed per unit biomass (Fig. 7), the shape of the graphs changed drastically between the two plant species. Nitrogen and P quantities were >100-fold greater



**Fig. 6.** Nitrogen and phosphorus concentrations in 5-year-old *P. caribaea* and *S. leratii* as a function of the dosage of sludge amendment. Within each graph, columns with the same letter are not significantly different at  $P=0.05$ . Capped lines are  $\pm$  s.e.



**Fig. 7.** Quantities of nitrogen and phosphorus as a function of the dosage of sludge amendment. Within each graph, columns with the same letter are not significantly different at  $P=0.05$ .

in *P. caribaea* than *S. leratii*. Considering *P. caribaea*, the quantity of N and P increased with sludge application rate. By contrast, the quantity of N and P in *S. leratii* increased with sewage sludge dose for the first three doses, and then decreased with the two highest doses.

#### Available soil phosphorus

Five years after sludge application, the amount of soil P potentially available for the plant showed two main trends (Fig. 8). The first comprises the four treatments with 2–10 mg P kg<sup>-1</sup>. The second trend was for the sludge treatment of 30 t DW ha<sup>-1</sup>, with ~80 mg kg<sup>-1</sup> of P potentially available for plants, which was ~8 times higher than with the other amendments. This treatment had a significant effect on soil P availability.

Clearly from Figs 6 and 8, a good correlation exists between the concentration of P in plants and the potentially available soil P extracted with Olsen's modified Dabin's method.

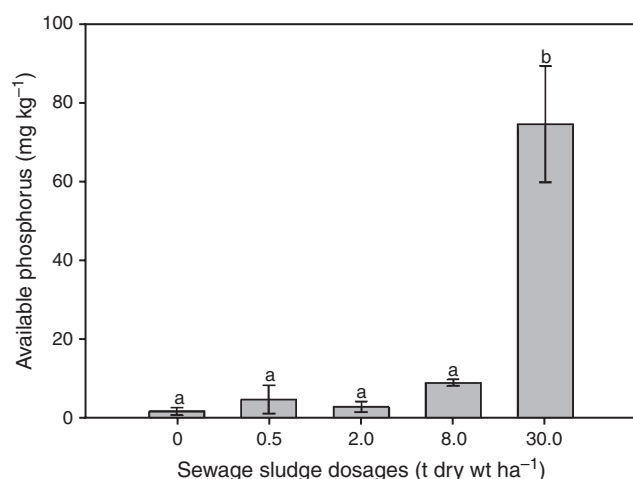
#### Carbon content in pines

The carbon concentration in the aboveground biomass of *P. caribaea* ranged between 48% and 51% of the dry weight (Fig. 9). The level of carbon in *P. caribaea* was greater in stems than in other compartments. In branches, when this compartment was relevant, carbon levels were lower than in either foliage or stems. Furthermore, the amount of carbon stored in the form of biomass was negligible for the first three treatments, whereas for the last two the quantities were high. The maximum amount stored in plants in the treatment 8 t DW ha<sup>-1</sup> was 1.4, 0.6 and 0.2 kg m<sup>-2</sup> for stems, foliage and branches, respectively.

Pine carbon storage in biomass was ~2 t C ha<sup>-1</sup> for treatments of 0.5 and 2 t DW ha<sup>-1</sup> and ~20 t C ha<sup>-1</sup> for the two highest sludge treatments. With no sludge, carbon storage by pine trees was only 0.003 t C ha<sup>-1</sup>.

#### Plant heavy metal content

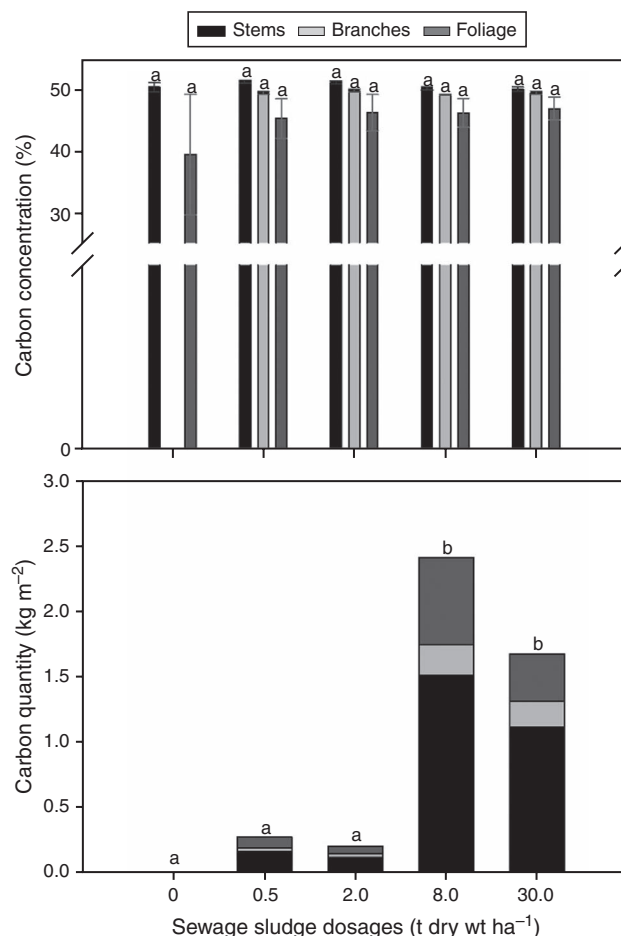
Trace element concentrations in the aboveground biomass (Table 3) are specified by treatment, by plant part, and in



**Fig. 8.** Available soil phosphorus versus sewage sludge dosage 5 years after spreading. Columns with the same letter are not significantly different at  $P=0.05$ . Capped lines are  $\pm$  s.e.

whole aboveground biomass. For the branch compartment, there were no data for the control plot as no branches had formed.

Trees from the control plot were very small and covered with earth dust. Despite repeated rinsing, a little dust always remained on the plants. Consequently, the geochemical signature of the soil influenced the metal analysis values, resulting in high amounts of Cr, Ni, iron (Fe) and aluminium (Al) in the small plants of the control plots. Accordingly, for this plot, only values from the two highest and least dusty trees were noted. Due to its low representativity, the control plot was not taken into account for statistical studies of metal distribution. Regarding the effect of sludge treatment on metal concentration in the different parts of the plants (stems, branches or foliage), no significant differences were observed for most trace metals. However, Al and Ni did present small differences; in foliage, Al concentrations were significantly higher in the 30 t DW ha<sup>-1</sup> treatment than in the two lowest treatments (0.5 and 2 t DW ha<sup>-1</sup>); for Ni, stem concentrations were significantly greater for the highest sludge treatment than for the others. Except for these two metals, no effect of sludge dose on metal



**Fig. 9.** Carbon concentration and carbon quantity in 5-year-old pine trees. Within each graph, columns with the same letter are not significantly different at  $P=0.05$ . Capped lines are  $\pm$  s.e.

**Table 3. Concentrations ( $\mu\text{g g}^{-1}$ ) of trace elements in pine trees 5 years after sludge application**

Values in italics do not include all data, only those from the most representative trees, and are not taken into account in analyses. Within a column, means followed by the same letter are not significantly different at  $P=0.05$ . n.d., Not determined

Appl. rate (t DW ha <sup>-1</sup> )	Mg	Al	Ca	Cr	Mn	Fe	Co	Ni	Cu	Zn	Ba
<i>Stem</i>											
0	<i>312</i>	<i>273</i>	<i>440</i>	<i>9.8</i>	<i>182</i>	<i>286</i>	<i>0.3</i>	<i>5.1</i>	<i>3.1</i>	<i>19.7</i>	<i>5.2</i>
0.5	340±35	105±9	309±46	1.8±0.5	26±4	66±12	0.1±0	2.9a±0.7	3.0±0.4	8.1±1.3	2.1±0.7
2	572±8	105±14	221±20	2.3±0.7	34±7	86±17	0.1±0	2.5a±0.9	4.2±1.1	11.5±1.6	4.3±1.6
8	502±76	115±11	330±43	1.9±0.9	22±3	115±65	0.1±0	2.0a±0.6	2.5±0.2	9.9±1.3	2.4±0.8
30	467±98	140±30	254±49	2.1±0.7	40±19	87±22	0.2±0.1	10.3b±3	4.3±2.2	10.9±2.2	2.9±0.7
<i>Branch</i>											
0	<i>n.d.</i>	<i>n.d.</i>	<i>n.d.</i>	<i>n.d.</i>	<i>n.d.</i>	<i>n.d.</i>	<i>n.d.</i>	<i>n.d.</i>	<i>n.d.</i>	<i>n.d.</i>	<i>n.d.</i>
0.5	393±75	104±16	373±46	3.7±1.1	63±8	93±20	0.1±0	4.7±1.6	5.3±1.2	14.2±2.1	3.7±1.1
2	534±76	122±51	341±25	4.8±3.8	43±5	172±120	0.2±0	4.9±1.3	5.7±1.9	11.9±1.8	1.7±0.4
8	548±79	138±26	513±43	1.3±0.2	78±21	69±8	0.2±0	4.2±0.7	7.2±1.6	18.0±1.4	4.3±1.0
30	554±87	153±51	407±91	0.9±0.2	62±20	71±27	0.1±0	10.3±3.4	2.6±0.5	15.2±2	3.8±1.0
<i>Foliage</i>											
0	<i>387</i>	<i>299</i>	<i>995</i>	<i>3.2</i>	<i>545</i>	<i>132</i>	<i>0.3</i>	<i>7.4</i>	<i>2.2</i>	<i>21.3</i>	<i>3.2</i>
0.5	554±108	193a±16	706±47	1.5±0.2	231±34	69±6	0.2±0	9.4±2.1	1.9±0.2	20.4±3	4.8±3.1
2	823±121	222a±25	789±88	1.6±0.3	181±27	104±28	0.3±0	5.2±1.4	3.3±1.1	16.0±1.7	2.3±0.5
8	694±106	274ab±31	784±111	1.7±0.2	156±19	121±35	0.2±0	7.9±1.7	3.7±1.5	18.8±1.8	5.1±1.3
30	677±176	332b±49	799±183	1.8±0.3	189±26	80±10	0.2±0	10.1±3	3.4±0.6	23.8±2.9	4.9±1.2
<i>Whole plant</i>											
0	<i>335</i>	<i>281</i>	<i>606</i>	<i>7.8</i>	<i>291</i>	<i>240</i>	<i>0.3</i>	<i>5.8</i>	<i>2.8</i>	<i>20.2</i>	<i>4.6</i>
0.5	411±70	130±11	428±56	1.7±0.4	86±8	68±7	0.1±0	4.9±1.6	2.9±0.4	12.1±1.4	3.2±0.9
2	606±152	135±5	385±9	2.0±0.1	69±10	88±17	0.2±0	3.4±0.5	3.7±1.2	12.8±1.6	3.3±1.2
8	463±61	147±11	458±38	1.5±0.6	63±5	92±31	0.2±0	3.9±0.4	3.5±0.1	12.2±1.4	3.4±0.4
30	485±68	177±28	432±55	2.1±0.3	78±15	73±15	0.2±0	8.2±3.2	4.5±2.3	15.7±2.4	3.6±0.2

absorption was evident. Due to contamination with the soil, control plot values showed higher values.

Regarding the effect of plant compartment on metal concentration for the different sludge treatments, no significant differences were observed for Mg, Cr, Fe, Ba or Cd. For Al, Ca, Co, Mn, Zn and Ni, the foliage concentrations were usually significantly greater than the concentrations for all sludge treatment levels. For Cu, differences were not as clear.

Two-way ANOVA performed only on the amended plots indicated that combined effect (treatment × plant compartment) had no effect on plant metal absorption. For metal concentrations in the whole plant, the highest were found in the control plot where contamination with soil may occur. Some trace elements appeared to slightly increase with sludge treatments (Al, Fe, Co, Ni, Zn, Ba); nevertheless, no significant differences were established.

## Discussion

In this study, seeds from the topsoil were left to germinate spontaneously. The composition of the resulting plant cover depended on the dosage of sewage sludge applied; for the lowest doses, the soil still presented low fertility and was favourable for native species, the main ones being *S. leratii* and *C. comosa*. By contrast, for the highest levels of application, the soil was greatly enriched and more favourable to exotic species suited to these conditions. Consequently, *P. caribaea* and *Paspalum* sp. predominated, and although native species germinated they were unable to develop.

The density of successfully germinated pine seedlings decreased as amendment increased. The salts contained in the sludge, leading to high electrical conductivity, might have a negative effect on germination as previously shown by Myers and Couper (1989). Although the control plot presented higher pine densities, mortality was higher but decreased when the soil was amended. Selivanovskaya and Latypova (2006) showed that sludge application decreased the mortality of *Pinus sylvestris*. However, increased mortality has been observed for *P. echinata* and *P. taeda* (Berry 1977) and adult *P. caribaea* (Watanabe *et al.* 2009). This effect was attributed to competition through the growth of herbaceous plants leading to water depletion.

In the current study, *P. caribaea* growth was greatly improved with amendment by sludge. Other authors have demonstrated improvement of pine growth following application of organic amendments: *P. sylvestris* (Selivanovskaya and Latypova 2006), *P. radiata* (Kimberley *et al.* 2004; Wang *et al.* 2004) and *P. halepensis* (Bastida *et al.* 2007). In the present study, the growth of *P. caribaea* was low in the control plots, indicating that this low-nutrient soil was not suitable for the natural development of this species at this site. After 5 years on the control plots, the mean height of the pine trees was just ~15 cm. As demonstrated in the experiment of Srivastava *et al.* (1979) and Maghembe and Redhead (1984), this corresponds to the height of a 6-month-old *P. caribaea* grown in pots with NPK mineral fertiliser.

In the present study, the growth rate of *P. caribaea* was obviously improved by sludge application, reaching 1.1 m



year<sup>-1</sup> at the highest dose. These values are in accordance with results from plantation experiments (Srivastava *et al.* 1979; Maghembe and Redhead 1984; Arias *et al.* 2011). Whereas previous experiments were mainly conducted with inorganic fertilisers and transplanted trees, our study concerned spontaneous revegetation and organic amendment in a single application. Wang *et al.* (2006) reported 1.75 m year<sup>-1</sup> in an experiment with a plantation receiving two sewage sludge amendments (600 kg N ha<sup>-1</sup>). For a 6-year-old *P. caribaea* stand, Arias *et al.* (2011) reported 1.7 m year<sup>-1</sup> but in very fertile Andic soil. According to those authors, growth rates were more dependent on soil conditions and quality than on genetic variability.

Sewage sludge improved tree growth and, consequently, biomass production. In the present study, the productivity of the pines with the highest levels of application was ~8–10 t ha<sup>-1</sup> year<sup>-1</sup>, which is slightly under values reported for this species. Egunjobi and Bada (1979) and Arias *et al.* (2011) obtained aboveground biomass of 10–12 t ha<sup>-1</sup> year<sup>-1</sup>. Ambagahaduwa *et al.* (2009), with data from the literature, developed a polynomial relationship estimating the *P. caribaea* biomass with stand age. The model takes account of available literature data (15 species of pines trees) and is therefore mainly based on plantations receiving inorganic fertilisation. Large variations may be observed due to location, altitude, rainfall, stand management, tree densities. Nevertheless, the estimated value from this polynomial model was 8 t ha<sup>-1</sup> year<sup>-1</sup>. In fact, pine productivity from a self-regenerated system using sewage sludge may result in the same productivity range as a planting system. From this biomass, the stem represents the major compartment of the tree (nearly 60%). This is in accordance with data usually reported in the literature for this species, where the trunk represents 70% of the total aboveground biomass for a 6–10-year-old *P. caribaea* population (Egunjobi and Bada 1979). The same values were obtained by Arias *et al.* (2011) for trees >14 m high. For smaller trees (<8 m), the stem biomass only represents 50% of the total aboveground biomass. The proportion of stem thus seems to increase with the height of the trees. In our study, foliage (needles) represented the second compartment of the aboveground biomass (30%). This was not the case in the study of Arias *et al.* (2011) where the proportion of foliage was <10%. The organic amendment used here seems to be favourable for pine foliage development.

*Pinus caribaea* and *S. leratii* nutrient contents were greater in needles than in stem and branches. For N, the needles presented the highest levels of nutrient whatever the amendment level. For P, only the highest level of amendment presented a significantly higher concentration in needles (near 0.2%). This nutrient distribution was also in accordance with literature values focussing on plantations (Egunjobi and Bada 1979; Kadeba 1994; Bramryd 2002; Wang *et al.* 2004), whereas the current study concerns passive restoration.

The P contribution to *P. caribaea* growth may be linked to a deficiency in this nutrient. As presented in Güsewell (2004), for the terrestrial ecosystem, N:P ratios (w:w) <10 and >20 correspond to N- and P-limited plants, respectively. In the current study the N:P ratio of the sewage sludge was ~2.5, which means that P was in excess with respect to N. In our

control experiment, the N:P ratio in whole pine plants was near 28. This value therefore corresponds to P-limitation of tree growth. A sludge application 0.5–8 t DW ha<sup>-1</sup> resulted in whole-tree N:P values of ~15. The trees thus do not seem limited for either of these nutrients. With the highest level of amendment (30 t DW ha<sup>-1</sup>), the P input was relatively high and pine trees became N-limited (N:P=5). This may explain how the plots with the highest level of amendment still showed available P higher than with the other treatments even 5 years after spreading. Wang *et al.* (2004), for *P. radiata* amended with sewage sludge, observed N:P ratios <9, which indicates borderline N limitation, in contrast to most other studies. Srivastava *et al.* (1979) and Manikam and Srivastava (1980) observed for *P. caribaea*, in plastic pots, that P was the only element that promoted height gain. According to those authors, the most striking effect was achieved with a contribution of 330 kg P ha<sup>-1</sup>, resulting in a growth of 30 cm height in 6 months. In our field experiment, for a similar dosage but of organic form, the effect of P was confirmed on growth from sludge application at 8 t DW ha<sup>-1</sup>. Phosphorus is an essential nutrient and may be insufficient in ultramafic soils, where it is immobilised by ferrous oxides, reducing its availability for plants. However, Gavalda *et al.* (2005) found that, in an Alfisol, the P-extractable content was enhanced by sludge supply and this was observable in our study for the highest dosage where P was in excess. This is interesting because the current study concerns a soil containing much more iron oxide than the soil used in Gavalda *et al.* (2005).

The nutrient content in pine trees implies nutrient storage in the biomass. For Egunjobi and Bada (1979), this storage in the total aboveground biomass of 6-year-old *P. caribaea* was 1 g P and 22 g N m<sup>-2</sup>. For the stems only, those authors found 0.4 g P and 10 g N m<sup>-2</sup>, similar to levels reported by Arias *et al.* (2011) of 0.5 g P and 11 g N m<sup>-2</sup>. These data were in accordance with our values for applications of 8 and 30 t sludge ha<sup>-1</sup>, with organic input for passive restoration compared with mineral fertilisation and planting.

In the current study, an average of 50% of the tree biomass was stored carbon, which is in agreement with literature values; the stem, the main part of the aboveground biomass, presented the highest carbon content, followed by branches and needles. Arias *et al.* (2011) observed the same distribution in several trees including *P. caribaea*. For those authors, carbon storage in their pine specimens (50 years old) was 74 t C ha<sup>-1</sup>. For *P. sylvestris* (a 69-year-old stand; Janssens *et al.* 1999) and *P. radiata* (a 16-year-old stand; Guo *et al.* 2008), mean carbon storage was ~90 and 75 t C ha<sup>-1</sup>, respectively. For *P. ponderosa*, Laclau (2003) estimated 44 t C ha<sup>-1</sup> of carbon storage in the aboveground biomass at different sites (15–20-year-old stand); moreover, the author observed that the total carbon storage in biomass was dependent on the total rainfall at the stand. As at our experimental site rainfall was sufficient (1800 mm year<sup>-1</sup>), and the total carbon storage in pine biomass depended on the nutrient supply to the soil. With no amendment, carbon storage was negligible, whereas for the highest sludge treatments, mean carbon storage in 5-year-old *P. caribaea* was estimated at 15–20 t C ha<sup>-1</sup>. For the same species, in an 18-year-old stand, Shin *et al.* (2007) estimated aboveground carbon storage at 60–130 t C ha<sup>-1</sup> depending on the stand soil quality. These values represent mean annual storage of

3–7 t C ha<sup>-1</sup>, i.e. 15–35 t C ha<sup>-1</sup> for a 5-year-old stand. These values are in accordance with our experimental measurement, implying that using sewage sludge with spontaneous regrowth results in as much carbon storage as in a pine plantation. Thus, sewage sludge may be considered as assisting carbon storage in biomass, as recently reported for farm manure (Ghosh *et al.* 2012).

Soils derived from serpentines are especially elevated in Cr, sometimes to >100 000 mg kg<sup>-1</sup>. Despite the very high Cr soil concentration, this element was poorly available, only 2 µg g<sup>-1</sup> in whole plant compared with values from Kabata-Pendias (2011), who found higher concentrations of Cr in pine trees in the Ukraine: 4 mg kg<sup>-1</sup> for older needles, 1.6 mg kg<sup>-1</sup> for branches and 0.3 mg kg<sup>-1</sup> for wood. Effectively, in ultramafic soils, Cr occurs mainly in the insoluble phases. However, organic substances added in the sewage sludge to the soil caused a significant increase of two Cr species, one associated with hydrous oxides and the other bound to organic matter (Kabata-Pendias 2011). That may explain why Cr concentration was very low in plants. Regarding plant effects, Cr is not subjected to specific uptake but Cr compounds were reported highly toxic to plants and detrimental to their growth and development (Shanker *et al.* 2005).

In the present study, concentrations were greater in plants of the control plots, so there was probably an effect of dilution in the biomass that masked concentration changes and especially an effect of the 'soil dust' stuck to small trees. Moreover, our values correlated with those of Wang *et al.* (2004), who demonstrated that the sludge did not appear to increase absorption of metals by plants. There were two exceptions: for aluminium, where values were dose-dependent, and for iron, where our values were higher, but not dose-dependent. However, the values found for iron were similar to those found by Latham and Verliere (1973) for *P. caribaea* in New Caledonia. Compared with that study, all values were in the range showing no particular effects from sewage sludge.

Kabata-Pendias (2011) gave approximate concentrations of trace elements in mature leaf tissues. Compared with their values, values below normal were found for Cu (5–30 mg kg<sup>-1</sup>) and Zn (27–150 mg kg<sup>-1</sup>) and normal values were found for Mn (30–300 mg kg<sup>-1</sup>). For Cr concentrations, values found were above normal (0.1–0.5 mg kg<sup>-1</sup>) but below excessive (5–30 mg kg<sup>-1</sup>). Cobalt concentrations were considered normal (0.02–1 mg kg<sup>-1</sup>) and Ni concentrations between normal (0.1–5 mg kg<sup>-1</sup>) and toxic (10–100 mg kg<sup>-1</sup>).

## Conclusions

This study, performed on a Ferralsol in Southern New Caledonia, showed that although the application of sludge decreased the potential of seeds from the soil storage bank to germinate, those that did emerge showed much improved growth.

The germination established durable plant cover, essential for the prevention of erosion. The application of sludge led to an increase of nutrient availability (N and P) for the development of plants and consequently of plant biomass. For P, with the three lower doses, the sludge effect was observed only for plants, but with higher doses, it was observed for plants and also for soil.

For the highest dose, P was in excess in the plant with respect to N, and consequently present in large quantities in the soil. For the ratio N:P, the optimum sludge supply leading to the maximum biomass without any P excess in soil should be slightly below 30 t DW ha<sup>-1</sup>.

Carbon storage in the biomass produced was increased at least 10-fold by the nutrient in the organic amendment spread on the land. So, sludge amendment may be used to promote trapping of atmospheric carbon. Sludge does not seem to affect the levels of metals absorbed by plants, even for Cr, which was present at high levels in the soil.

However, the application of abundant organic amendment encouraged the growth of undesirable exotic species. From these results, the optimal dose of sewage sludge depended on the objectives. The results of the current study provide valuable information to support the use of sewage sludge on New Caledonian soil. To stimulate biodiversity, optimal dosage seems to be 0.5 or 2 t DW ha<sup>-1</sup> but to reach maximal biomass, the best level of application is 8–30 t DW ha<sup>-1</sup>, probably ~20 t DW ha<sup>-1</sup>.

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