The University of Reading

Department of Soil Science

Leaf-cutting ants (*Atta colombica*) and soil biogeochemistry: Nests as islands of fertility

by

Theresa Hudson

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ABSTRACT

The traditional view of nutrient cycling in tropical forests is as an efficient, tightly controlled system where vegetation is the key influence. In the Neotropics, leaf-cutting ants are also seen to be an important factor in the distribution of nutrients. In this study, *Atta colombica* on Barro Colorado Island, Panama, are seen to have a large effect on nutrient cycling within a moist lowland tropical forest. Waste mounds represent "islands of fertility" and nest sites "islands of infertility", providing pulses of bioavailable nutrients in patches throughout the forest.

12 nest sites were sampled from, four active, four abandoned within one year and 4 abandoned more than one year ago. Soils were analysed for total nutrients and soluble nutrients extracted in $CaCl_2$ and also for identification and quantification of phosphorus compounds, determined by solution ^{31}P NMR spectroscopy. To gain insight into the ecological significance of *A. colombica* colonies, root biomass and nutrient content was also measured.

Waste material contains approximately 5 times greater concentrations of total carbon, nitrogen and phosphorus than control soils, and increases are also seen in soil under waste. In older nests (abandoned for more than one year), the level of nutrients is comparable with control soils. Increases in the concentration of soluble nutrients (phosphate, nitrate and ammonium) are even greater, and concentrations of ammonium and nitrate are still visible in nests that have been abandoned for several years. Analysis of phosphorus compounds show elevated concentrations of both inorganic and organic phosphorus in waste material and soil under waste and an indication that microbial activity is higher in these samples, even after several years after a nest is abandoned. Nutrient concentrations in nest soils are almost always lower than control soils, although this is not statistically significant.

Fine root biomass increases in waste gardens of abandoned nests and contains greater concentrations of nitrogen and phosphorus compared to fine roots in control soils. These changes are also seen in older nests, supporting the hypothesis that *A. colombica* activity is ecologically significant to the surrounding plant community and the ecosystem as a whole.

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1. INTRODUCTION

Described as 'ecosystem engineers' (Jones *et al*, 1994), ants are an integral part of many habitats. In the Neotropics, leaf-cutting ants, of which there are two genera; *Atta* and *Acromyrmex*, are a common feature in forests, savannahs and grasslands. Living in colonies with between 10^3 and 10^6 workers, they create nests with a surface area of up to 50-160 m^2 (Farji-Brener & Silva, 1995b). They live in symbiosis with a saprophytic basidiomycete fungus, *Leucoagaricus* or *Leucocopriuns*, which is cultivated in subterranean chambers. Leaves, fruits and flowers from a variety of plant species are harvested by the ants and metabolized by the fungus, which in turn feeds the colony. A single *Atta* colony can harvest 1-2 tonnes of fresh plant material each year (Folgarait, 1998). Such a high level of activity inevitably results in a large volume of waste being produced. Organic material that cannot be metabolized by the fungus, secondary plant compounds, dead ants and fungus as well as parasitic fungal mycelia are all disposed of (Wirth *et al*, 2003) either in nest chambers, used by most leaf-cutting ant species, or, for the species *Atta colombica*, in an external waste mound.

External waste mounds are placed up to 12 m from the nest entrance and can be over 2 m in diameter (Hart & Ratnieks, 2002). Almost half are placed in streams or ponds, at the base of a tree or next to a rock, fallen tree or liana. This allows worker ants to have as little contact with the waste as possible, to minimise contamination of the nest with fungal parasites and toxic bacteria present in the waste (Hart & Ratnieks, 2002; Wirth *et al*, 2003). Waste mounds are always located downhill from a nest for this purpose, to avoid toxins washing into the nest chambers following rainfall.

A colonys' activities have wide ranging effects on an ecosystem. Foraging and nest building result in the movement of both plant material and soil, causing spatial heterogeneity (Wagner *et al*, 1997). *Atta sexdens* moves up to 20 tonnes of soil to create a nest covering 100 m^2 (Folgarait, 1998). Excavation on this scale changes various physical properties of the soil. In many ant species, nest building results in lower bulk density in nest soils, increased moisture content, aeration and drainage and alterations in texture (Folgarait, 1998; Wagner *et al*, 1997; Mandel & Sorensen, 1982; Petal, 1998; Moutinho *et al*, 2003; Wagner, 1997).

The cultivation of fungus gardens and the harvesting of large volumes of plant material into the nest lead to chemical changes in the soil. Nest soils of seed

harvesting ants, *Pogomonyrmex* sp., contain high concentrations of organic matter, phosphorus, potassium and mineral nitrogen compared to surrounding soils (Wagner *et al*, 1997). This is also seen in the nests of *Formica polyctena* (Kristiansen & Amelung, 2001), *Formica perpilosa* (Wagner, 1997), *Atta sexdens* (Moutinho *et al*, 2003) and *Atta laevigata* nests, which saw an increase in the concentration of nitrogen, calcium, magnesium and organic carbon (Farji-Brener & Silva, 1995b; Farji-Brener & Silva, 1995a). These changes are also seen in many termite species, such as *Cubitermes* sp. (Roose-Amsaleg *et al*, 2005). There is a greater effect on soil nutrient concentration from nests built on poorer soils (Folgarait, 1998), where small scale changes such as these can be referred to as "islands of fertility" (Schlesinger *et al*, 1996), providing much needed nutrients in less fertile ecosystems.

The largest changes occur in the waste that is created; nutrients are concentrated in waste material and incorporated into the soil. In most species, waste material is accumulated in detrital chambers within the nest and therefore changes in nutrient concentration vary with depth. Movement of soil caused by Atta species are seen down to 5 m below the soil surface (Moutinho *et al*, 2003) and waste chambers tend to be at the lowest point so as not to leach toxins into non-waste chambers. Nitrate levels in the nests of *Atta Sexdens* increase with depth, with the largest concentrations being in the waste material (Verchot *et al*, 2003). Increased nitrogen, but not carbon, in detrital chambers leads to a lower C:N ratio than surrounding soils.

Atta colombica differs from other ant and other leaf-cutting ant species, due to the existence of external waste mounds, which is where nutrient changes are seen, not in nest soils. Concentrations of many elements, including phosphorus, potassium, calcium, sodium, sulphur and copper are all greater than in surrounding soils (Haines, 1978).

The accumulation of nutrients continues as a nest ages. In species that do not relocate nest sites such as *Pogonomyrmex barbatus*, the concentration of organic matter, total and mineral nitrogen and orthophosphate continues for decades (Wagner *et al*, 2004; Wagner *et al*, 1997). Older nests of *Formica polyctena*, because of decomposition processes, contain less of the accumulated carbon and nitrogen after abandonment, although there is still more in nest soils compared to surrounding soils more than 20 years after abandonment (Kristiansen & Amelung, 2001). Leaf-cutting ants relocate nest sites frequently, up to 25% of the total population move each year (Wirth *et al*, 2003). Such transitional behaviour means that the changes in nutrient

cycling are likely to be shorter lived and the effect can be over a much larger area than just one nest.

Decomposition in ant nests is more rapid than in surrounding soils because of a greater abundance of soil fungi and bacterial populations (Folgarait, 1998). Higher numbers of some micro-organisms usually leads to higher levels of predation, which can mobilise nutrients in the biomass (Wagner *et al*, 1997). Microbial activity is thought to be increased in the soil near the detrital chambers and the fungus gardens (Verchot *et al*, 2003). N mineralization rates in soils from *Formica perpilosa* nests were higher than in surrounding soils, indicating increased microbial activity (Wagner, 1997). It was not known whether the increase in microbial activity results in a higher diversity of micro-organisms or simply a greater abundance. Roose-Amsaleg *et al* (2005) measured higher organic matter and inorganic phosphorus fractions in soil-feeding termite nests along with increased enzymatic activity, concluding that increased nutrient and moisture content led to an increase in bacterial and fungal growth. This is not seen in all species, however. Nests of *Pogonomyrmex barbatus* do not contain a greater abundance of fungi and bacteria species (Wagner *et al*, 2004). *Formica polyctena* accumulate organic matter in nests, but the turnover rate is slower than in other species (Kristiansen & Amelung, 2001) suggesting that there is not a significant increase in microbial activity.

The accumulation of nutrients and the alteration of the microbial community in the soil can affect plant growth and success. Vegetation is removed from the nest area when active, which is maintained by the ants, partly through continual excavation of soil onto the top of the nest (Farji-Brener & Illes, 2000). Active nests of *Atta cephalotes* reduce understorey plant diversity, although this effect is reversed after abandonment, when diversity and abundance of understorey plants is much higher than in surrounding areas (Garrettson *et al*, 1998). Abandoned nests provide areas of forest free from competition for light and a concentrated patch of soil nutrients. Farji-Brener & Illes (2000) discuss the idea that *Atta* species create “bottom-up” gaps in the forest; in the same way that tree fall creates “top-down” gaps. The high turnover rate of nests, the modifying of soil nutrients, the clearing of nest vegetation and the acceleration of nutrient cycling all support this theory and suggest that the changes caused by Atta colonies are large enough to instigate plant response.

Leaf-litter ants, *Pheidole* sp, in lowland tropical forest in Costa Rica concentrate seeds in waste mounds in the nest, providing an ideal medium for

seedling establishment (Levey & Byrne, 1993). This is also true of *Atta laevigata* nesting in parkland savannah in Venezuela (Farji-Brener & Silva, 1996). It is also thought that they help determine the tree: grass ratio, increasing the abundance of deciduous trees on the savannah (Farji-Brener & Silva, 1995b).

Patchy nutrient concentration can lead to proliferation of roots by plants to acquire the excess nutrients (Jackson & Caldwell, 1996; Haines, 1978). A greater number of fine roots are seen in the nests of *Atta sexdens*, due to increased nutrients and also because the soil is more penetrable and therefore easier for roots to grow into (Moutinho *et al*, 2003). *Atta cepahlotes* nests have been seen not to increase fine root growth (Farji-Brener & Medina, 2000) and, in Amazonia, increases in root biomass in *Atta sexdens* nests did not result in increased tree diameters (Moutinho *et al*, 2003).

Nutrients in tropical forests are known to be very tightly cycled, the majority being held in the biomass (Vitousek & Sanford, 1986). Mineralization rates in the soil are high and it would be expected that any 'islands of fertility' in the forest are rapidly exploited by vegetation, leaving soil relatively low in nutrients. Soils in this study have a low level of extractable phosphorus, even for tropical soils (Yavitt *et al*, 1993). Fumigation-released phosphorus is in higher concentrations than most tropical soils, suggesting that microbes compete successfully with plants for available phosphorus, and any processes that can aid plants in acquiring phosphorus could be of great importance. In terms of nitrogen, the soil is moderately fertile (Yavitt *et al*, 1993).

Previous studies on *Atta colombica* looked at total nutrients only, resulting in an incomplete picture as to the alterations to nutrient cycling. Sampling was carried out during the dry season, when the ants are less active, and so underestimating the effect they have on the nutrient status of the soil (Haines, 1978). I look at the nests of *Atta colombica*, how they change the nutrient content of the soil in both the nest and waste mounds and how this effect changes with time after nests have been abandoned. Total and soluble nutrients are analysed along with phosphorus composition by solution ^{31}P NMR spectroscopy with the view of giving a more complete picture of nutrient cycling influenced by leaf-cutting ants. In order to assess the effect of *Atta colombica* colonies on vegetation, root biomass and root nutrients are analysed, with the aim of concluding that *Atta colombica* create "islands of fertility" in the forest and impact the surrounding plant community.

2. MATERIALS AND METHODS

2.1 Study Site

Barro Colorado Island (BCI) is situated in Gatun Lake in Panama ($9^{o}09'$N,$79^{o}51'$W). Originally a hilltop, it became an island when the lake was formed artificially during the creation of the Panama Canal. The mean annual temperature is 26°C, which varies by only 1°C on a monthly basis. Mean annual rainfall is 2600 mm with a four month dry season from December to April. The entire 1564 ha island is covered with semi-deciduous tropical moist forest. Elevation ranges between 20 and 160 m above sea level.

There are three main types of geology on BCI: an andesite cap, a marine limestone, and a volcanic mudflow deposit. The andesite cap has weathered to form a clay-rich, Yellow-Brown Oxisol, while the limestone and volcanic deposits have formed silty-clay, Yellow-Brown Alfisols (Yavitt, 2000).

The ant species studied was *Atta colombica* (Guerin-Meneville 1844), whose colonies comprise up to five million worker ants and one queen. The nest structure consists of underground nest chambers containing fungus gardens and a conspicuous waste dump away from the nest. Waste is always deposited down slope from the nest (Figure 1).

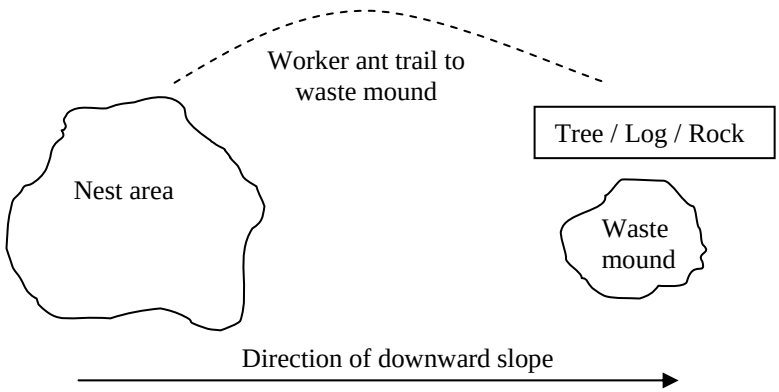

Figure 1: Schematic plan view of a typical nest site.

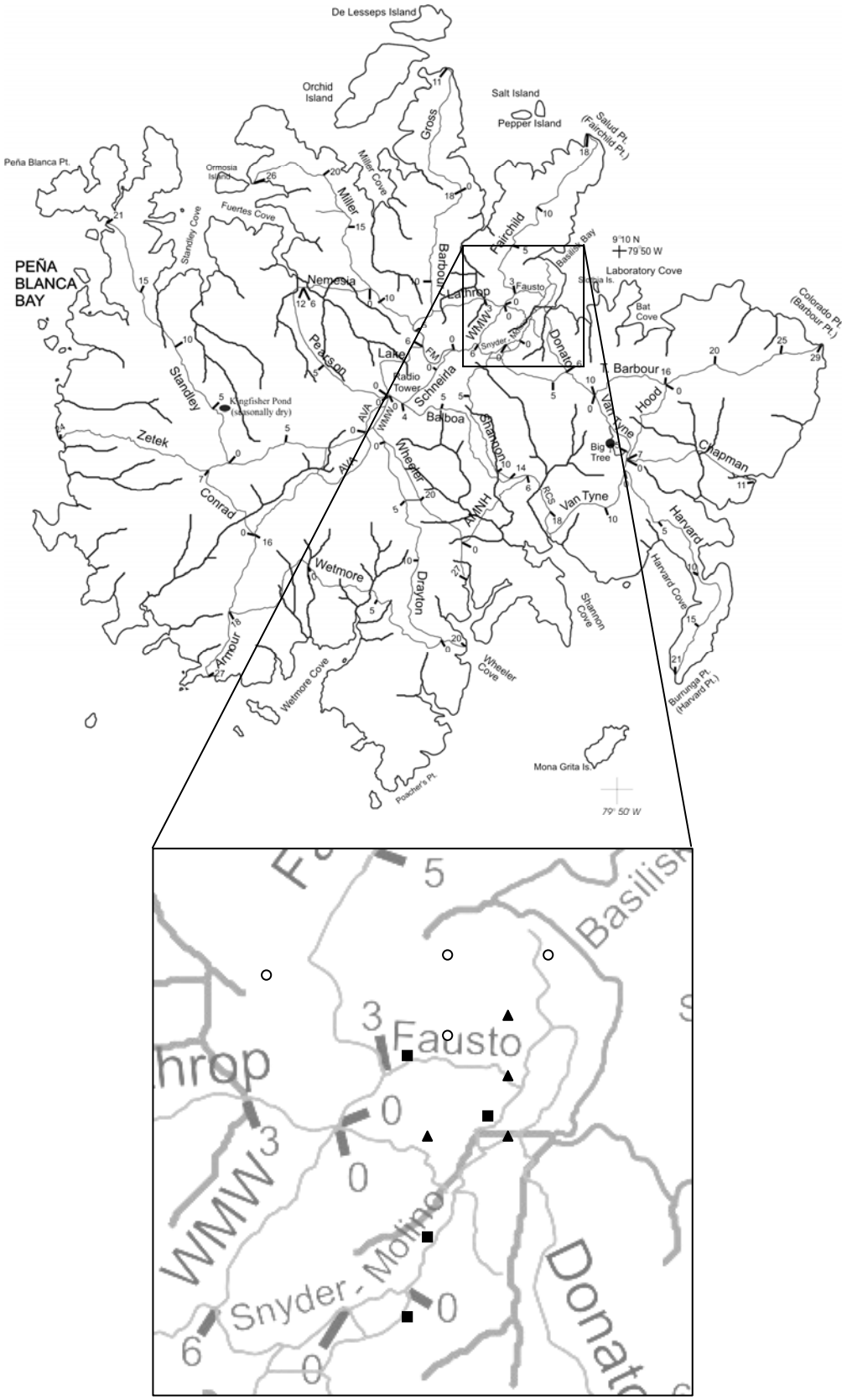

Figure 2: Map of Barro Colorado Island (1564 ha), showing nest locations. ▲ = Active nests; ■ = abandoned <1 year ago; ○ = abandoned >1 year ago.

Twelve nest sites were identified: four active nests, four that had been abandoned within one year and four that had been abandoned between one and three years previously. Nests were identified from long term (12 year) records of ant nest dynamics maintained by Hubert Herz, Smithsonian Tropical Research Institute. They were chosen for their age and proximity to each other to minimise other environmental variables. Location of nests can be seen in figure 2.

2.2 Sampling

Sampling took place in May and June 2005, at the beginning of the wet season. For each nest, soil was taken from the nest, from an area adjacent to the nest (acting as a control sample), from the waste garden, and the soil under the waste garden. Waste mounds from six of the abandoned nests had completely decomposed and so soil was sampled from where the waste garden had been located and was classified as soil under waste. A diagram of a typical nest site is shown in Figure 2.

Soil for chemical analysis was taken using a trowel to 10 cm depth, ensuring an even core of soil was removed. Three cores were taken from each area of the site (nest, non-nest, waste and soil under waste) and bulked, totalling around 500 g of soil per bulked sample. Non-nest soil samples were chosen up slope from the nest, between 2 and 5 metres away from the nest. The samples were sealed in Ziploc® bags and refrigerated until analysis.

Root biomass and root nutrients were measured in all of the abandoned nests and one of the active nests. Three replicate cores were taken from nest soil, non-nest soil, waste gardens and soil under the waste using a 10 cm diameter soil corer with a drop weight to a soil depth of 10 cm.

2.3 Laboratory Analysis

The moisture content of fresh soil was determined gravimetrically by oven drying at 105°C for 14 h. Soil pH was measured in 0.01 M $CaCl_2$ (1:4 soil to solution ratio) using a Thomas Scientific Model 8005 pH electrode.

For determination of total carbon (C) and nitrogen (N), samples were dried to 60°C and ground using a ball mill grinder. The ground samples were analysed for total C and N on a FlashEA 1112 Series NC soil analyser.

All nitrogen (N) and phosphorus (P) analysis was carried out on a Lachat QuikChem® 8500 automated ion flow injection analyser (except ^{31}P NMR Spectroscopy). Ground samples were used for measuring total P via acid digestion (Kjeldahl method) and the supernatant analysed. Fresh samples used for extractable N and P analysis were shaken with 0.01 M $CaCl_2$ in a 1:4 soil to $CaCl_2$ ratio and then vacuum filtered through Whatman GF/A glass microfibre filter papers. Nitrate, phosphate and ammonium were measured using molybdate colorimetry with detection at 880nm for phosphate, sulphanilamide reaction after reduction to nitrite using a cadmium catalyst with detection at 520nm for nitrate and salicylate-nitroprusside reaction with detection at 660nm for ammonium.

For ^{31}P NMR spectroscopy, P was extracted by shaking 5 g of soil with 100 ml of 0.25 *M* NaOH and 0.05 *M* EDTA solution for 16 h. These were then centrifuged at 10,000 x *g* for 30 minutes and half the supernatant frozen at -80°C. Total P was measured in the rest of the supernatant, to calculate the recovery of P from the NaOH-EDTA extraction. The frozen extract was lyophilized and ground to a fine powder. Each freeze-dried extract (~100 mg) was re-dissolved in 0.1 mL of deuterium oxide and 0.9 mL of a solution containing 1 *M* NaOH and 0.1 *M* EDTA, then transferred to a 5-mm NMR tube. The deuterium oxide provided an NMR signal lock and the NaOH raised the pH to >13 to ensure consistent chemical shifts and optimum spectral resolution. Inclusion of EDTA in the NMR tube reduces line broadening by chelating free Fe in solution.

Solution ^{31}P NMR spectra were obtained using a Bruker Avance DRX 500 MHz spectrometer operating at 202.456 MHz for ^{31}P and 500.134 MHz for ^{1}H. Samples were analyzed using a 6 µs pulse (45°), a delay time of 1.0 s, and an acquisition time of 0.4 s. Between 16,000 and 28,000 scans were acquired depending on the P concentration of the lyophilized extract, and broadband proton decoupling was used for all samples. Chemical shifts of signals were determined in parts per million (ppm) relative to an external standard of 85% H_3PO_4. Signals were assigned to individual P compounds or functional groups based on literature reports (Turner et al., 2003) and signal areas calculated by integration. Spectra were plotted with a line broadening of 5 Hz.

Root biomass samples were washed through a 50 µm sieve and the retrieved roots separated into three size classes; fine roots (<2mm diameter), small roots (2-5mm diameter) and large roots (>5mm diameter). They were then dried for 48 h at 60°C for the determination of root biomass. The fine root samples were ground using a ball mill, digested (Kjeldahl method) and analysed for total N and P as ammonium and phosphate respectively, using the same methods as for the soil samples.

2.4 Statistical Analysis

Soil data was $\log_{10}$ transformed after being tested for normality to meet parametric assumptions. Root data did not need to be transformed. Split plot factorial analysis was carried out on the data using SAS 9.1 with repeated measurements for correlation between type (nest soil, waste, under waste and control). 1-way ANOVA was used to analyse the waste for soil nutrients and root data and also for active nests in root data, as the number of samples for these differed to the remainder of the data set.

3. RESULTS

3.1 Soil Moisture and pH

The mean pH in $CaCl_2$ for control (non-nest) soils was 5.3. The largest pH values were found in the active waste mounds, with a mean of 6.2. Smallest values were measured in nest soils, with a mean of 4.9. There was no significant difference in pH between nest sites or within nests ($p>0.05$).

Waste samples in active nests had higher moisture contents than other samples. All other moisture content values ranged between 29.9% and 52.1%, although there were no significant differences between age of nest or type of sample ($p>0.05$).

3.2 Soil Total Nutrients

The amount of total C, N and P was significantly higher in the waste mounds than in soils (C: $F=57.14$, $p<0.001$; N: $F=52.21$, $p<0.001$; P: $F=47.58$, $p<0.001$). This difference was greater in active nests than recently abandoned nests, where levels in waste samples were around five times that of the non-nest soil (Figure 3). There was significantly more C, N and P in the soil under waste than in nest and control (non-nest) soils (C: $F=14.81$, $p<0.0001$; N: $F=15.55$, $p<0.0001$; P: $F=19.65$, $p<0.0001$). In active and recently abandoned nests, levels in nest soils were less than in control (non-nest) soils, although this was not significant. Control soils contained similar amounts of C, N and P, for all sites. In older nests (>1 year abandonment), the levels of C, N and P were similar in control, nest and soil under waste ($p>0.05$).

There are no differences in C:N ratios between different ages of nest or within nest sites (Table 1). There is more variation in N:P and C:P ratios, with the greatest change being between nest soils in recently abandoned (<1 year) and long abandoned (>1 year) sites. There is an increase in both the C:P and N:P ratios as nests age, meaning less P in relation to both N and C in nest soils in older nests.

Table 1: Mean element ratios in soils and waste material for all ages and type of sample with errors in italics.

		C:N	N:P	C:P
Active	Waste	11.1 *(0.5)*	11.9 *(9.7)*	135.3 *(116.4)*
	Soil under waste	10.4 *(0.9)*	10.0 *(4.1)*	101.8 *(33.7)*
	Nest soil	10.9 *(1.1)*	8.0 *(2.2)*	88.0 *(29.4)*
	Control (non-nest) soil	11.0 *(3.8)*	6.4 *(2.8)*	67.8 *(31.3)*
<1 year	Waste	11.0 *(0.9)*	13.1 *(7.9)*	146.2 *(103.0)*
	Soil under waste	10.7 *(1.1)*	13.3 *(2.9)*	143.4 *(26.5)*
	Nest soil	10.8 *(1.1)*	5.1 *(1.3)*	54.8 *(12.8)*
	Control (non-nest soil)	10.7 *(1.0)*	6.5 *(1.5)*	69.8 *(20.0)*
>1 year	Soil under waste	10.4 *(0.6)*	12.8 *(6.5)*	134.7 *(71.1)*
	Nest soil	11.1 *(1.4)*	14.7 *(7.7)*	155.5 *(66.2)*
	Control (non-nest soil)	10.3 *(0.4)*	8.6 *(1.9)*	88.9 *(21.8)*

3.3 $CaCl_2$ Extractable Nutrients

Soluble nitrate levels were significantly higher in waste material and soil under waste material in both active nests (F=22.01, p<0.001) and recently abandoned nests (<1 yr) (F=22.82, p<0.001) (Figure 4). Soil under waste contained significantly more nitrate than both nest and control (non-nest) soils at all ages and nest soils contained less than control soils, also at all ages (F=18.91, p<0.0001). Age significantly lowered nitrate levels on all types of sample except control soils (F=18.47, p<0.001).

There were significantly higher levels of soluble ammonium in the waste material in both active and recently abandoned (<1 year) nests (F=11.36, p<0.001). Soils under waste contained more ammonium than control or nest soil (F=42.59, p<0.0001) and this difference was seen even in older nests.

Waste material also contained significantly more soluble phosphate than all other samples, although this was true only of active nests (F=10.23, p<0.05). Older waste, nest, control, and under-waste soils all contain very low, often undetectable levels of phosphate (despite a limit of detection of only 0.004 mg P kg^{-1} soil).

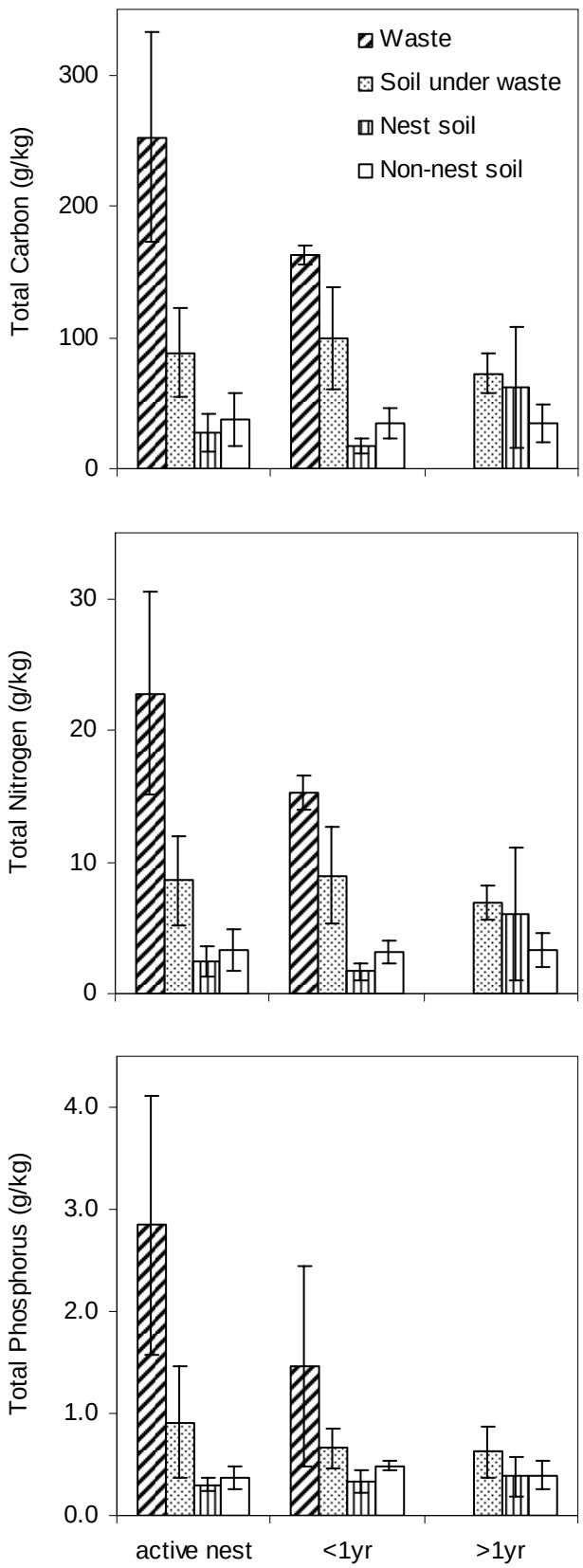

Figure 3: Mean total C, N and P values for all ages and type of sample. Non-nest soil samples act as the control for each site.

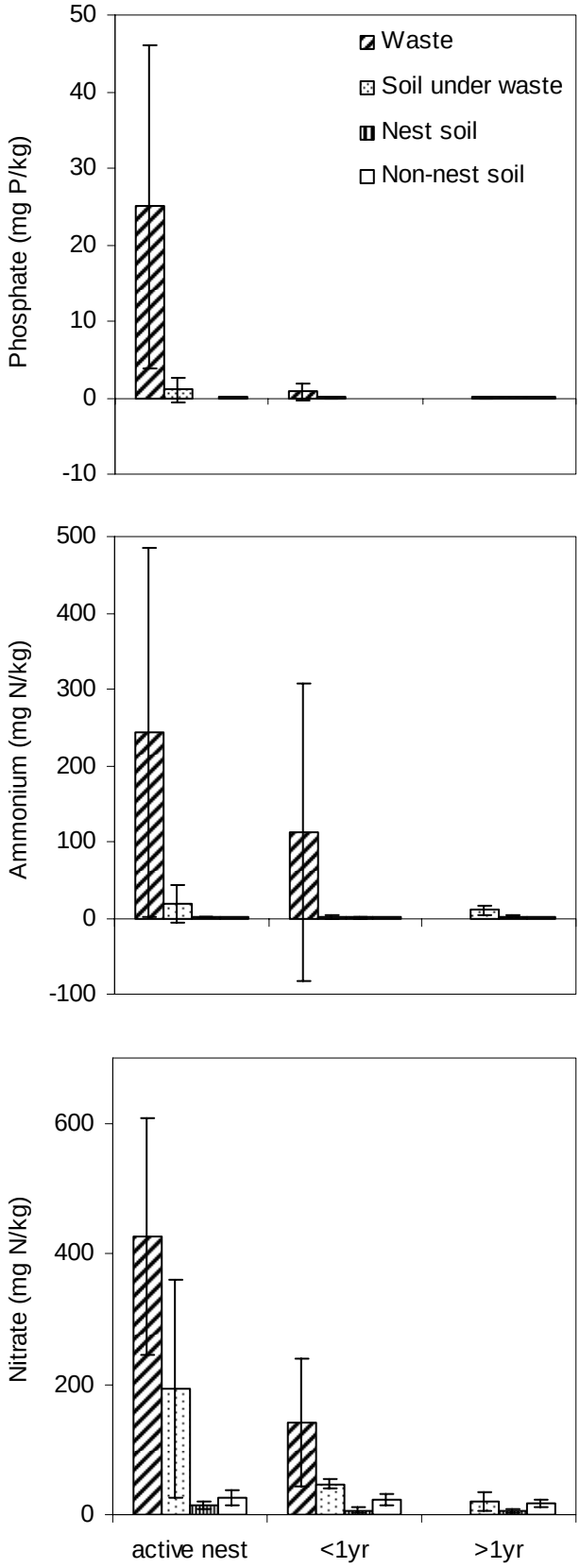

Figure 4: Mean PO_4^{3-}, NO_3^- and NH_4^+ values for all ages and type of sample. Non-nest samples act as the control for each site.

3.4 Phosphorus Composition by Solution ^{31}P NMR Spectroscopy

Spectra resulting from the NMR analysis are shown in Figure 5. The 0.25 *M* NaOH / 0.05 *M* EDTA solution extracted between 122.77 and 1609.97 mg P kg^{-1} from the different samples. These amounts represent between 32 and 108% soil total phosphorus (Table 2). Soils under waste gave the highest recovery, with a mean of 79%. Nest soils gave the lowest recovery, with a mean of 42%. This was similar to the control (non-nest) soils, which gave a mean recovery of 48%. The two waste samples were very different, with 56% P recovery from active waste and 81% recovery from waste in abandoned nests.

Table 2: Concentration of P extracted in 0.25 *M* NaOH / 0.05 *M* EDTA solution and % recovery of total P.

		NaOH – EDTA extracted P (mg P kg^{-1} soil)	% Recovery
Active	Waste	1609.97	56
	Soil under waste	788.97	108
	Nest soil	167.97	56
	Control (non-nest) soil	232.97	59
<1 year	Waste	892.97	81
	Soil under waste	434.97	76
	Nest soil	129.37	39
	Control (non-nest soil)	224.97	46
>1 year	Soil under waste	371.97	60
	Nest soil	122.77	32
	Control (non-nest soil)	158.97	40

Waste material contained a much larger proportion of inorganic P (phosphate, pyrophosphate, polyphosphate) compared to the nest and control samples in both active and recently abandoned (<1 year) nests (Table 3). Soil under waste in active nests also contained more inorganic P. Waste samples contained more organic P (phosphonate, phosphate monoesters, DNA and phospholipids) than soil samples, while soil under waste contained more organic P than nest and control soils.

Waste samples contained more phosphate and phosphate monoesters than any other samples. This was also true of soil under waste, which contained more than both nest and control (non-nest) soils. The amount of phosphate and phosphate monoesters

in both the waste and soils under waste decreased with the age of the nest, but was still greater than the control soils. Phosphate levels in the waste material halved during the first year of abandonment, although this was still 10x the amount measured in control soils. Both waste and soil under waste samples of active nests were the only samples to contain more phosphate than phosphate monoesters.

DNA was also most prevalent in waste samples, double the amount measured in the control soils and was also higher in soil under waste. Nest soils contained similar levels to control soils. DNA levels did not decrease as the age of the nest increased. Phospholipids were only detected in waste samples and soil under waste samples, although not soil under waste in recently abandoned nests.

Concentrations of pyrophosphate were highest in waste samples. Soil under waste also contained more than the control. Polyphosphate was only detected in active waste and the control soils of older nests. Phosphonate was not detected in any of the waste or soil under waste samples, although trace amounts were detected in nest and control soils. Nest soils consistently contained less of all P compounds than control soils, regardless of the age of the site.

There is also a compound at 3.5 ppm (Figure 5) in waste and soil under waste samples which is most likely to be glucose-1-phosphate.

Table 3: P compounds (mg P/kg soil) and percentage organic / inorganic P in 11 bulked samples of each sample type and each age group. ND= not detectable, TR= trace.

	Active				<1 year				>1 year		
Phosphorus compound	Waste	Under waste	Nest	Non -nest	Waste	Under waste	Nest	Non-nest	Under waste	Nest	Non -nest
	mg P kg^{-1} soil										
Phosphonate	ND	ND	ND	5	ND	ND	TR	TR	ND	TR	TR
Phosphate	1099	527	51	56	511	150	32	58	110	35	48
P-monoesters	330	189	90	131	266	239	72	137	190	58	74
DNA	66	35	20	30	50	34	14	21	34	25	23
P-lipids	44	20	ND	ND	41	ND	ND	ND	21	ND	ND
Pyro-P	33	17	7	10	25	13	12	9	16	5	7
Poly-P	37	ND	ND	ND	ND	ND	ND	ND	ND	ND	7
Inorganic P	1170	544	58	67	536	162	44	67	127	40	62
Organic P	440	245	109	166	357	273	86	158	245	83	97
	% NaOH-EDTA extracted P										
Inorganic P	27	31	65	71	40	63	66	70	66	67	61
Organic P	73	69	35	29	60	37	34	30	34	33	39

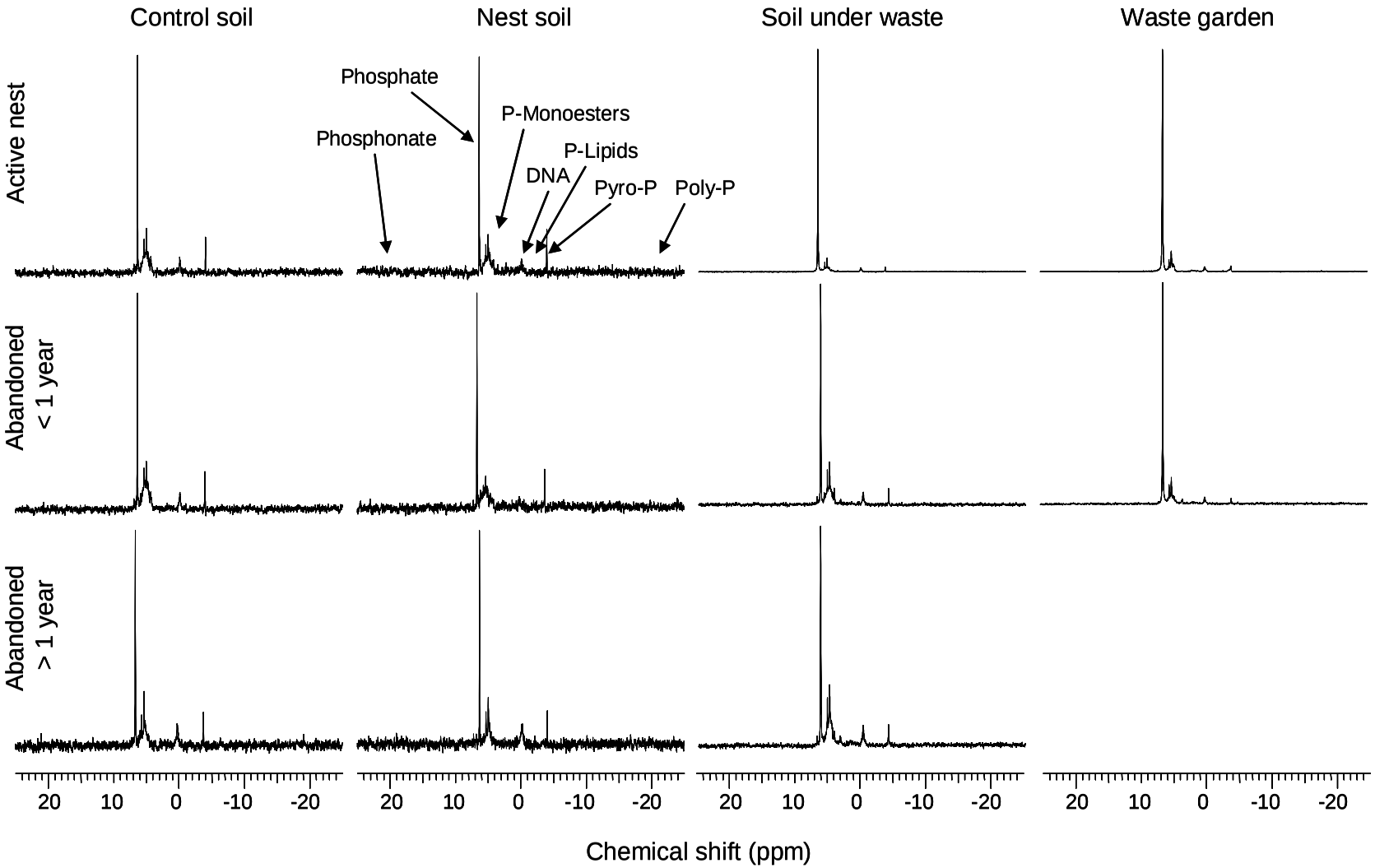

Figure 5: Solution ^{31}P Nuclear Magnetic Resonance spectra for 11 bulked samples. Labels show at which point in the spectra certain P compounds are measured.

3.5 Root Biomass and Root Nutrients

In active nests, waste samples contained significantly less fine root biomass than other samples (F = 11.51, p < 0.003), which were all similar. In recently abandoned nests (<1 year), waste material and soil under waste contained more fine root biomass than both nest and control soil (F=15.68, p<0.0001). Also in recently abandoned nests (< 1 year), nest soil contained significantly less fine root biomass than control soils. Older nests (abandoned > 1 year) generally contained more fine-root biomass than recently abandoned or active nests, although this was not the case compared to the control soil (Figure 6). Comparison with the control showed less fine roots in active nests, more fine roots in waste and soil under waste in recently abandoned nests, which became a smaller increase in older nests. Nest soils contained less fine roots than control soil in recently abandoned nests and, to a lesser extent, in older nests. There were no significant differences in root biomass in the 2-5 mm and >5 mm diameter root size classes.

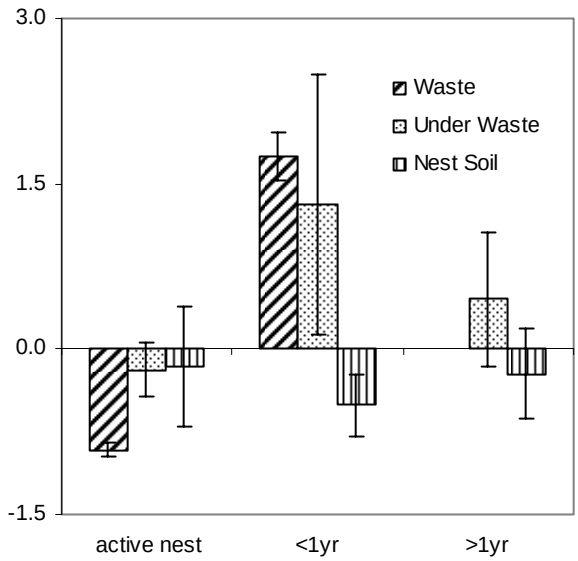

Figure 6: Fine root biomass in waste, under waste and nest soil standardised to the control (non-nest) soil.

For the active nest sampled for root biomass, there was a significantly lower P concentration in fine roots from the nest and a significantly greater concentration in fine roots from soil under waste (F=7.03, p<0.05) compared to the control (Figure 7).

The waste samples taken from recently abandoned nests contained elevated levels of nitrogen (F=11.44, p<0.001).

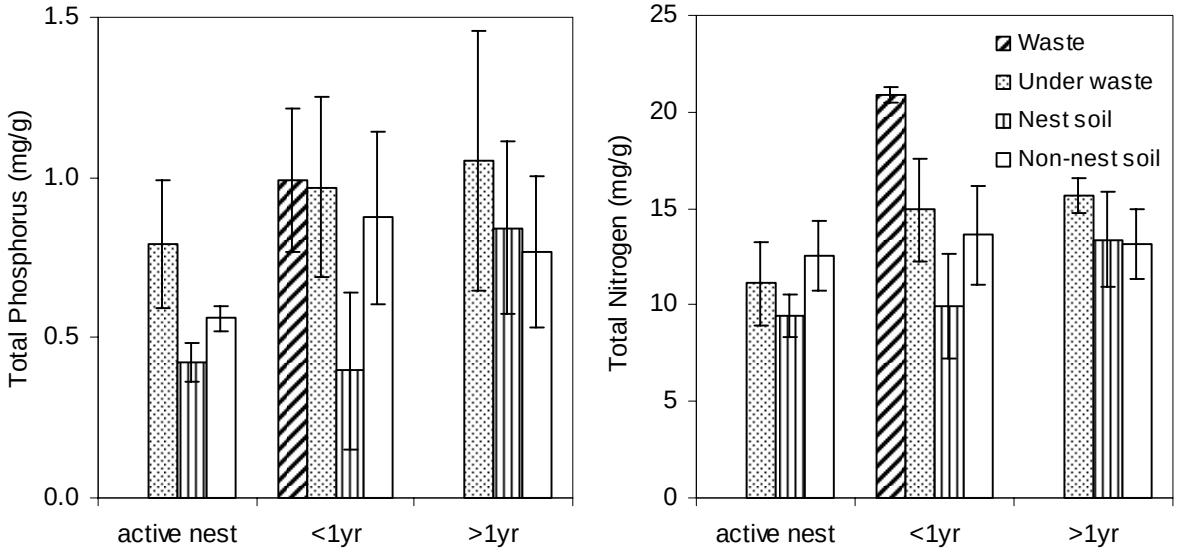

Figure 7: Mean fine root N and P concentrations. Non-nest soils are used as the control.

Both root N and P content in nest, control and under waste soils show significant interaction between both recently and long abandoned ages of nest (N: F=10.52, p<0.001; P: F=8.02, p<0.05). This is shown in the nest samples. Fine roots in recently abandoned nest soils contain less N and P than in control soils and soil under waste soils. In older nests, this difference becomes insignificant (p>0.05).

4. DISCUSSION

4.1 Total Nutrients

High concentrations of total C, N and P in the waste suggest that a large proportion of harvested plant material is not being utilised. This could be as high as 73-89% of harvested material being wasted (Abril & Bucher, 2004). Before leaves drop, the plant will remove nutrients from the leaf, which are then redistributed to other parts of the plant (Vitousek, 1984). *Atta* harvest young, growing, nutrient-rich leaves, channelling a lot more C, N and P through to the waste than is being deposited in control soils via litterfall. Previous studies on BCI have shown that N and P levels in waste material were higher than in fresh litterfall by a factor of 1.7 and 2.1 respectively (Haines, 1978). Whilst the nest is active, new material is constantly being deposited on the waste mound, giving a continual supply of organic matter and nutrients. After abandonment, changes to nutrient concentrations are relatively short lived. Nutrients in waste mounds from nests abandoned for longer than one year have mostly returned to background concentrations. This could be due to several factors, such as leaching of soluble nutrients (particularly nitrogen), rapid uptake by plants or adsorption into the soil matrix.

The slightly depressed concentrations in the nest soil are not significant, but are consistent in most samples. This could be caused by the formation of the nest, as soil is brought up from depth, which would contain lower concentrations of nutrients. As the nest area is kept vegetation free, less litter falls and there is less replacement of nutrients. It has also been suggested that the fungus may remove nitrogen from the surrounding soil to supplement what the ants provide, which would maintain a lower N concentration (Abril & Bucher, 2004).

The C:N ratio does not change significantly spatially or temporally, indicating that any excess C and N in the soil and waste mounds are used at the same rate, neither one being particularly limiting. C:P and N:P ratios increase slightly as the nests age, particularly in nest soils, suggesting P is lost or utilised more rapidly than C or N. Plant selectivity during foraging tends towards high-protein tissue, which would concentrate N in the nest (Wagner *et al*, 2004). The ratios show this not to be the case in this study, although the similar C:N ratio in the waste and control soils

may suggest that the fungus is utilising excess N and so the difference in ratio is not being seen in the waste.

An important point of interest is that a large amount of nutrients are not being utilised by the fungus and ants, considering the energy expenditure required in harvesting so much plant material. In terms of carbon compounds, only soluble carbon and phenols are metabolized by the fungus (Bucher *et al*, 2004). This is only a small proportion of the total carbon in the harvested plant material. Proteins, lignin, cellulose and other polymerized carbon compounds are not utilised by the fungus as they are unable to break them down, therefore giving high carbon concentrations in the waste (Abril & Bucher, 2002; Bucher *et al*, 2004).

Nitrogen from harvested leaves cannot be metabolized by the fungus, which would account for the large volume of nitrogen deposited in the waste mound. The fungus is protease deficient and requires the ants to secrete protease and nitrogen-containing compounds such as ammonia, amino acids, allantoin and allantoic acid (Martin, 1970). If not completely metabolized by the fungus, these would be removed to the waste mound.

4.2 $CaCl_2$ Extractable Nutrients

$CaCl_2$ extractable nutrients are highly soluble and plant available. Decreases in concentration are therefore likely to be due to uptake from plants, although some loss could occur from leaching. The loss of soluble phosphate in waste material is more rapid than total P, as it is only measured in the waste of active nests. There is not a significant increase in phosphate concentration in soil under waste, insinuating that excess available phosphate is being taken up by plants and not leached. The increased levels of phosphate in the soil are returned to control levels much faster than ammonium or nitrate. The reason for this is not clear. Phosphate could be adsorbed quickly to soil components such as clays and iron oxides, becoming insoluble, or could be taken up by plants more rapidly than ammonium or nitrate. This is verified by the nutrient ratio results.

Changes in soluble N, in the form of nitrate and ammonium are longer lived and show elevated concentrations even in the waste of nests that have been abandoned for longer than one year. Although most is removed by this stage, another limiting

factor could be inhibiting further uptake of N, for example available phosphate. This would imply that the control soil in this area of the forest is P, and not N, deficient, resulting in a greater demand for P.

When compared with harvested material, waste has more soluble nitrogen in both organic and inorganic forms (Bucher *et al*, 2004). Inorganic nitrogen comes from soil present in waste material, whereas the organic fraction is thought to come from secretions made by the ants onto the fungus garden or plant sap collected by the ants and consumed by workers. There is also the possibility that nitrogen fixation is occurring in the nest by nitrogen-fixing bacteria in the fungus garden (Haines, 1978; Bucher *et al*, 2004).

Mineralization and nitrification rates are higher in the waste chambers of *Atta sexdens* (Verchot *et al*, 2003). Ants can increase the abundance of ammonifying bacteria (Folgarait, 1998), speeding up the transformation of organic nitrogen in the soil, leading to a decrease in nitrogen levels in the soil.

4.3 ^{31}P NMR Spectroscopy

Results from the extraction in 0.25 *M* NaOH / 0.05 *M* EDTA solution represent all organic P and most inorganic P. A fraction of the inorganic P is not extracted (Turner *et al*, 2005). Nest soils contain lower concentrations of all P compounds, while waste and soil under waste contain more than the control soils, which is consistent with the total and soluble nutrient analysis. Elevated concentrations of phosphate monoesters in the waste material come from discarded plant material. Lower phosphate monoester concentrations in the nest soil are due to the ants clearing the vegetation, so no breakdown of plant material takes place. High concentrations of DNA and phospholipids are present in waste material and soil under waste, indicating high microbial activity. This does not diminish with increasing nest age, meaning that the increase in microbial activity in soil under waste is longer term than the increase in nutrients.

Previous analysis of this kind on *Formica polyctena* has shown that abandoned anthills are enriched with total P (Kristiansen *et al*, 2001). This enrichment was not just seen in the surface horizons of the soil profile, but continued throughout the profile. The relative distribution of P forms was not, however, seen to be altered by

the presence of ants. In this study, phospholipids are only detected in waste material and soil under waste, as is glucose-1-phosphate, which is rare in soil samples and is not found in the control soils on BCI.

As well as the plant available inorganic P measured in $CaCl_2$ extracts, the waste is also enriched with plant available organic P compounds, such as monoesters, including the degradation products of lipids and some inositol phosphates and diesters. Pyrophosphate can also be made available to plants, after hydrolysis by a phosphatase enzyme. In the waste of soil-feeding termites, phosphatase activity is higher than in surrounding soils (Roose-Amsaleg *et al*, 2005). If this is the case in *Atta colombica* waste mounds then pyrophosphate will be made available to plants.

As the nests age the phosphorus compounds diminish in the waste and soil under waste samples, although most are still at much greater concentrations than the control. Concentrations in the nest soil are still lower in older nests (abandoned >1 year) than in the control soils. It can therefore be shown that *Atta colombica* is having long term effects on the phosphorus concentration and composition of these soils, resulting in long term changes in microbial abundance and diversity and possibly changes in plant community composition and growth.

4.4 Root biomass and nutrient content

Within one year of a nest being abandoned, there are significantly more fine roots in the waste and soil under waste material. This is a positive sign that plants are taking advantage of these nutrient 'hotspots'. Fine roots provide an absorptive surface area, and biomass can be increased when there is a greater concentration of nutrients available. If existing plants are utilising the excess nutrients, then this would lead to an increase in the production of fruits and seeds, increased growth and general overall fitness (Farji-Brener & Medina, 2000).

Nest soils contain less fine roots than control soils. Vegetation is cleared from the nest area and there is mostly a lower concentration of nutrients in the soil, giving no incentive for plants to promote fine root growth into the nest soil. In nests that have been abandoned for longer than one year, there are still less fine roots than in control soils, but they have increased, matching the increase in nutrients seen in the nest soil samples as the nest ages. Increased root growth may be one reason why the waste is

placed away from the nest. Roots can have a damaging effect on the nest, as they take available water, which could leave the fungus garden water deficient, particularly during the dry season (Haines, 1978). This also explains why vegetation is removed from the nest area and few roots are found in the nest soils.

Fine root N and P concentrations follow the same trend as the soil nutrient concentration in nests, suggesting that not only are roots being produced in response to the increase in nutrient concentrations but that the uptake rate is greater in the additional fine roots than in existing roots. Even in the active nest sampled, root N and P concentration is higher in waste material and lower in nest soil than the control. This immediate response by plants is typical of the high nutrient turnover in tropical forest soils and provides an explanation as to why the “island of fertility” caused by the ant waste is so short-lived.

Nutrients in waste dumped against trees can be utilised by roots produced from the tree trunk above ground directly into the waste material (Figure 8). This provides a rapid uptake of the available nutrients and allows the tree to compete against seedling establishment in the waste, which is inhibited in areas of high fine root biomass as the competition for water is too great (Haines, 1978).

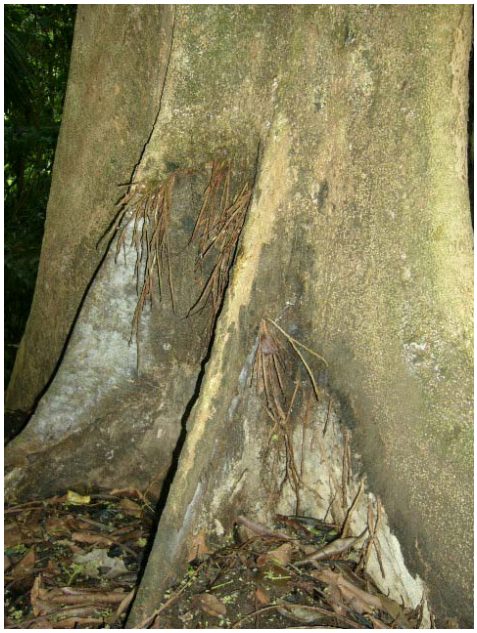

Figure 8: Tree roots growing directly into a waste mound, which has subsequently decomposed.

Once nutrient concentrations in the nest soils have returned to control levels, the area is an ideal location for seedling establishment. There is little competition and no litter to limit light availability (Hull-Sanders & Howard, 2003). These areas are similar to those caused by gaps from tree or branch fall. The transient nature of the ant colonies result in further similarities to traditional gap dynamics. It would be beneficial to the overall understanding of gap dynamics to further understand the effects of *Atta* colonies.

Waste material contains a higher number of seeds than surrounding soils, but the diversity is lower (Farji-Brener & Medina, 2000). This is probably due to the ant's species specific foraging behaviour. If seedling establishment is more successful on waste mounds and there is less diversity of seeds, then the resulting plant community may be less diverse than in areas without leaf-cutting ants.

4.5 The Role of *Atta colombica* in Ecosystem Functioning

Abundance of *Atta* colonies are correlated to the successional stage of the vegetation (Haines, 1978). The younger the vegetation, the greater number of colonies are found. At early stages of succession, a plant community is more susceptible to environmental influences, which will determine the climax community of the area. *Atta* colonies, therefore, are in a prime position to have a lasting influence on ecosystem structure.

External waste mounds encourage root growth from not only tree species, but shallower rooting shrub and herb species (Haines, 1978). As well as providing a source of available nutrients to existing plants, waste mounds provide ideal areas for the establishment of new seedlings, particularly in areas where the waste is not disposed of next to a tree. The establishment of seeds is most likely to be those left in the waste by ants, which for *Atta colombica* are species such as *Miconia* sp. (Wirth *et al*, 2003). Other leaf-cutting species with subterranean waste chambers will only provide available nutrients to tree species with deep root systems. The establishment of seedlings is unlikely as the waste is often too deep for successful germination and growth to occur. Seedlings on waste mounds are more likely to succeed over those on control soils as there is a richer supply of available nutrients. This signifies that the

ants are playing an important role in the composition of plant species that are being established on nest sites.

Species such as *Pogonomyrmex barbatus* remain at one nest site for over 20 years and continue to accumulate organic matter, N and P throughout the lifetime of the nest (Wagner *et al*, 2004). The transient nature of *Atta colombica* means that these "islands of fertility" are constantly being created in new areas. This results in a greater area of the forest being altered by a colony and also allows recolonisation of the abandoned nest site, leading to a much greater impact than a species which does not abandon nests so frequently.

Because of the low nutrient status of tropical forest soils, even the small increase in nutrient concentrations in soil under waste material in older nests could be important longer term changes in the soil. Because the accumulation of nutrients by the ants is patchy, "islands of fertility" are created in the forest. Nests, with slightly lower nutrient concentrations, could be considered as "islands of infertility", preventing recolonisation by species requiring higher concentrations of nutrients. Nest effects of *Atta cephalotes* on vegetation are only apparent on the nest site, with neighbouring areas being unaffected by the colonys' activity (Garrettson *et al*, 1989). This supports the idea that leaf-cutting ant activity increases heterogeneity in the forest, not just in terms of soil nutrients, but also in terms of plant community structure. This effect could be more wide-ranging than the nest site, as ants can travel hundreds of metres from the nest to forage and harvest around 10% of C and N in the foraging area (Wirth *et al*, 2003). Species that cannot survive this level of foraging pressure would eventually be replaced by species capable of replacing harvested leaves, or reacquiring lost nutrients. In areas such as BCI, where *A. colombica* abundance has dropped drastically over the past 3 or 4 years, future soil nutrient cycling will be altered, leading to changes in plant species structure which could in turn lead to changes in faunal composition.

5. CONCLUSION

This study has shown that *Atta colombica* interfere with soil nutrient cycles, which then has important consequences for root nutrient acquisition in moist tropical forests. This supports previous studies looking at this and other leaf-cutter ant species and their role in ecosystem functioning. Even a few years after abandonment, changes can be seen in soil chemistry and plant root activity where waste mounds were placed. Nest sites form a mixture of islands of fertility and infertility, creating microhabitats and increasing spatial heterogeneity. This has important ecological significance for plant community structure and helps to further understand biogeochemical cycling in tropical forests.

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