

# Seasonal Changes and Treatment Effects on Soil Inorganic Nutrients Following a Decade of Fertilizer Addition in a Lowland Tropical Forest

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We conducted monthly measurements of extractable soil nutrients, including N, P, base cations, and micronutrients, as well as the potential toxin Al, in a long-term fertilization experiment in lowland tropical rain forest in the Republic of Panama. Our prediction was that the response of individual nutrients to seasonal climate and fertilizer addition would vary depending on the nature of their biogeochemical cycles. We detected significant seasonal variation in soil pH and all nutrients measured, although only extractable K concentrations were greater in the early wet season, while extractable phosphate varied little in plots that did not receive P addition. A decade of N addition increased soil nitrate, had no effect on extractable ammonium, and decreased soil pH (~0.8 units in plots receiving only N). The decline in pH caused a corresponding decline in extractable base cations (Ca and K) and increased extractable Al, highlighting an important but poorly understood consequence of long-term atmospheric N deposition onto tropical forests. A decade of P addition increased extractable phosphate by 50-fold, indicating that chronic fertilizer addition has overcome the high phosphate sorption capacity of the soil. Potassium addition without N increased extractable soil K by 91%, but only by 25% when K was added in combination with N, suggesting that the previously reported N × K interactive effect on trunk growth rates could be a true response to N addition. Extractable Cu and Zn were increased twofold by micronutrient fertilizer addition, were reduced in the dry season, but were not affected by N addition (i.e., soil acidification). We conclude that the response of extractable nutrients to seasonal climate and fertilizer addition varies among nutrients, and suggest that greater attention be paid to the biological implications of acidification in response to long-term atmospheric N deposition onto strongly-weathered tropical forest soils.

Soil nutrient availability influences patterns of plant productivity and species distribution in tropical forests (Gentry, 1988; Clinebell et al., 1995; Palmiotto et al., 2004; John et al., 2007; Condit et al., 2013). Phosphorus is often considered to be the most important limiting nutrient in lowland tropical forests (Vitousek, 1984; Cleveland et al., 2011; Condit et al., 2013), based in part on the predominance of strongly-weathered soils in the lowland tropics and the characteristically low concentrations of extractable P that they contain (Walker and Syers, 1976; Johnson et al., 2003). However, the availability of N, base cations, and micronutrients can also influence the performance of tropical trees (Stark and Jordan, 1978; Vitousek, 1984; Cuevas and Medina, 1988; Barron et al., 2009; Wright et al., 2011). For example, long-term fertilizer addition to a lowland tropical forest in Panama, the site of the current study, showed that addition of P, N, and K each altered some aspect of forest productivity: P addition increased fine litter

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fall, K addition decreased fine root growth, and N plus K ameliorated a long-term decline in tree growth rates (Wright et al., 2011). In the same experiment, P and K both increased growth, photosynthesis, and rates of herbivory at the seedling stage (Pasquini and Santiago, 2012; Santiago et al., 2012), whereas K, P, and micronutrients increased litter decomposition rates (Kaspari et al., 2008). These results highlight the importance of understanding factors regulating the availability of multiple nutrients in lowland tropical forest soils (Townsend et al., 2011).

Seasonal patterns in nutrient availability are expected to occur in tropical forests with an extended dry period (Lodge et al., 1994), notably as a pulse of nutrients at the onset of the wet season (Singh et al., 1989; Cornejo et al., 1994; Yavitt and Wright, 1996; Campo et al., 1998; McGrath et al., 2000). A number of mechanisms could contribute to this. For example, much of the annual litter fall in seasonal forests occurs in the dry season and accumulates until rainfall begins (Wieder and Wright, 1995), so the onset of the wet season induces nutrient mineralization (and perhaps microbial immobilization) from decomposing litter. Phosphorus and other nutrients can be released from the soil microbial biomass by cell lysis when rapid rewetting follows an extended dry period (Sparling et al., 1985; Srivastava, 1997; Turner and Haygarth, 2001). Soil drying also changes soil physical and chemical properties that can influence extractable nutrient concentrations (Birch, 1960; Bartlett and James, 1980; Tack et al., 2006), although the effects appear to vary among soils and nutrients (Turner and Romero, 2009).

Here we report results from a detailed assessment of extractable soil nutrients, including P, N, base cations, and micronutrients, throughout an annual cycle in the tenth year of a long-term fertilization experiment in lowland tropical forest in the Republic of Panama. The experiment is well studied, with detailed information on plant productivity and soil properties (Kaspari et al., 2008; Koehler et al., 2009; Corre et al., 2010; Wright et al., 2011; Yavitt et al., 2011; Sayer et al., 2012). The forest is not deciduous, but leaf litter fall peaks during the dry season, when approximately 10% of tree species become leafless (Wieder and Wright, 1995). Although large-scale nutrient manipulations have been conducted in other sites (e.g., in tropical montane forests in Hawaii; Hall and Matson, 2003), there are very few in lowland tropical forests and the experiment in Panama is the only one to have included K and micronutrient treatments.

The present study had two objectives. First, we aimed to assess seasonal changes in extractable soil nutrients across an annual cycle, including the influence of nutrient enrichment on any seasonal patterns. Second, we sought to quantify the influence of a decade of fertilizer addition on extractable soil nutrients. This addresses issues raised after 4 yr of fertilization (Yavitt et al., 2011), such as (1) the extent to which a single mid-season sampling is representative of the entire annual cycle, and (2) whether the increase in extractable nutrients after the onset of fertilization reflects a rapid change to a new equilibrium or an intermediate step in a longer-term reorganization of soil nutrient cycles.

We predicted that the response of extractable nutrients to seasonal climate and fertilizer addition would vary among nutrients, based on differences in their biogeochemical cycles. For example, N is relatively transient in tropical forest soils, with large inputs through biological fixation and atmospheric deposition (Hietz et al., 2011; Reed et al., 2011) and large losses through leaching and denitrification (Houlton et al., 2006; Koehler et al., 2009; Corre et al., 2010). Similarly, K is mobile in the plant–soil system, being rapidly leached from leaves and retained only weakly in the soil (Schreeg et al., 2013b). We therefore expected N and K concentrations to increase moderately but rapidly to a new steady state under chronic fertilizer addition, and to show pronounced seasonality. In contrast, P is stabilized in soils through interactions with metal oxides, particularly in strongly-weathered soils with high P fixation capacity such as those at our study site (Schreeg et al., 2013a). We therefore expected extractable P concentrations to increase substantially but relatively slowly under chronic fertilizer addition as P sorption sites become progressively saturated. Phosphorus availability is also regulated strongly by microbial activity, which promotes rapid phosphate turnover through the soil solution (Oberson and Joner, 2005). We therefore expected seasonal patterns in extractable P to be muted by microbial cycling and buffering of the extractable phosphate pool by the relatively large total P pool (both inorganic and organic) (Frossard et al., 1995; Oberson and Joner, 2005). Micronutrients such as Cu, Mn, and Zn are stabilized in soil on cation exchange sites, including on clays, metal oxides, and organic matter (Jenne, 1968; Adriano, 1986), but concentrations are not buffered in the same manner as exchangeable P. We therefore expected extractable micronutrient concentrations to be increased by fertilizer addition and show seasonal variation associated with soil chemical changes linked to drying (Tack et al., 2006).

## MATERIALS AND METHODS

### The Gigante Fertilization Experiment

The study was conducted on the Gigante Peninsula (9° 06' 31" N, 79° 50' 37" W), part of the Barro Colorado Nature Monument, Republic of Panama. Elevation ranges from 25 to 61 m above sea level and the site supports mature moist semi-deciduous rain forest at least 200 yr old (Wright et al., 2011). On nearby Barro Colorado Island, the mean annual temperature is 26°C and annual rainfall averages 2600 mm, with just 10% falling during the four month dry season between December and April (Windsor, 1990). Soils are Oxisols developed on Miocene basalt and are morphologically similar to the Typic Eutrudox (AVA and Marron soil classes) on nearby Barro Colorado Island (Dieter et al., 2010). Topsoil texture is clay throughout the experiment, with no significant differences in sand, silt, or clay among treatments ( $p > 0.10$ ). Average values across all plots ( $\pm$  one standard error,  $n = 32$ ) were: clay ( $< 0.002$  mm)  $73.7 \pm 2.2\%$ , silt (0.002–0.05 mm)  $12.4 \pm 2.1\%$ , sand (0.05–2.0 mm)  $13.9 \pm 1.3\%$  (determined by the pipette method following pretreatment by  $H_2O_2$  to destroy organic matter and Na-dithionite to dissolve Fe oxides (Gee and Or, 2002)).

The chronic fertilization experiment began in 1998 and is described in detail elsewhere (Kaspari et al., 2008; Wright et al., 2011; Yavitt et al., 2011; Sayer et al., 2012). The experiment is remarkable in its duration and in the range of responses observed in terms of forest productivity, with significant effects involving N, K, and P (discussed above; Wright et al., 2011). Briefly, fertilizer is added four times per year in the wet season to 40 × 40 m plots replicated four times for each treatment in a factorial N-P-K design. Nitrogen is added as coated urea ((NH<sub>2</sub>)<sub>2</sub>CO), P as triple superphosphate (Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub>·H<sub>2</sub>O), and K as KCl. Annual doses are 125 kg N ha<sup>-1</sup>, 50 kg P ha<sup>-1</sup>, and 50 kg K ha<sup>-1</sup>. A further treatment (four replicate plots) includes Ca and Mg (from dolomitic limestone; 230 kg ha<sup>-1</sup>yr<sup>-1</sup>) and micronutrients (Soluble Trace Element Mix; The Scott's Miracle-Gro Company, Marysville, OH). The latter is applied at 25 kg ha<sup>-1</sup>yr<sup>-1</sup> and includes H<sub>3</sub>BO<sub>3</sub>, CuSO<sub>4</sub>, FeSO<sub>4</sub>, MnSO<sub>4</sub>, ZnSO<sub>4</sub>, and Na<sub>2</sub>MoO<sub>4</sub>. Fertilizer was added in equal amounts at the following times during the study period: 13–15 September 2006, 16–18 May 2007, 4–6 July 2007, 22–24 August 2007, and 10–12 October 2007 (Fig. 1). Fertilizer cannot be added during the dry season, because the granules do not dissolve under dry conditions.

The four replicates are placed perpendicular to the 36-m topographic gradient because soil properties parallel the gradient (Yavitt et al., 2009). Within each replicate, we blocked the N, P, K and NPK treatments versus the NP, NK, PK and control treatments. This balanced, incomplete-block design minimizes uncontrolled error associated with spatial variation, enables evaluation of main effects and two-way interactions, but limits evaluation of the three-way interaction. To preclude movement of nutrients among plots, the minimum distance between plots was 40 m, except for two plots separated by 20 m and a 3-m deep streambed.

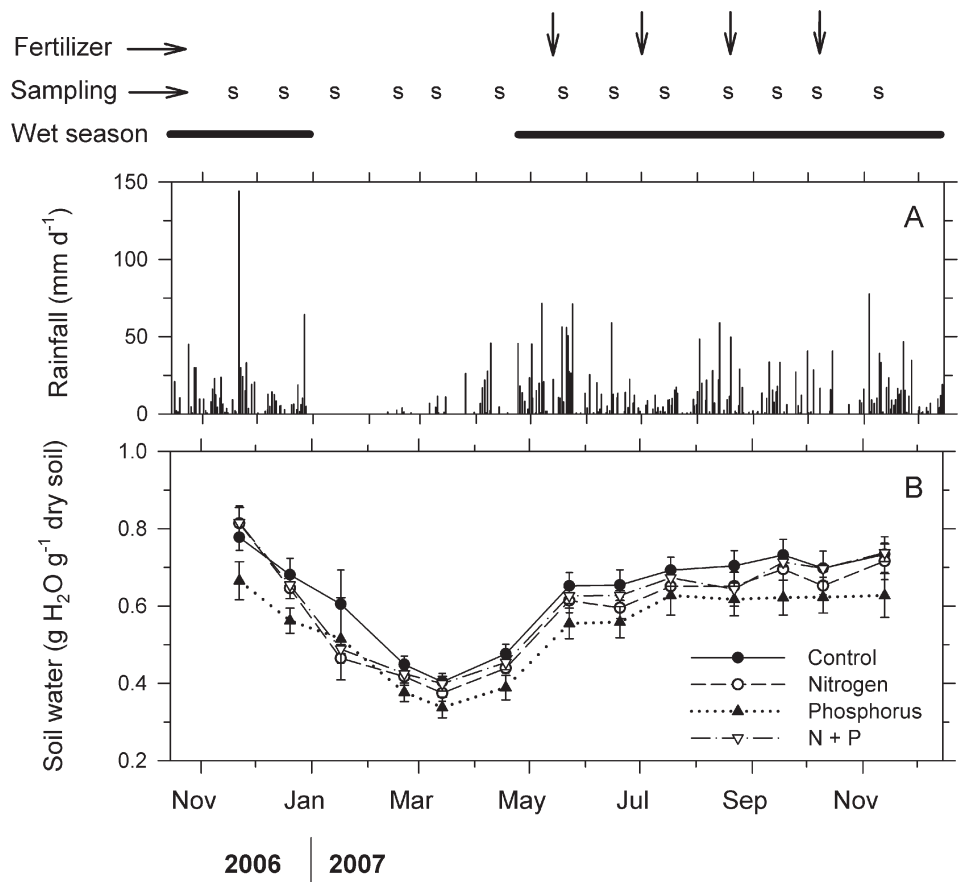
### Climate during the Study Period

Rainfall was measured at 5 min intervals at the Smithsonian Tropical Research Institute meteorological station on Barro Colorado Island, ~ 6 km from the study site, and summed to calculate daily totals. Soil temperature at the Gigante site during the study period was reported previously (Koehler et al., 2009).

### Soil Sampling

Soils were sampled between November 2006 and November 2007 (Fig. 1). During the third week of every calendar month, soils were collected from 16 plots in an N × P factorial design (control, +N, +P, +NP). Every 4 mo all 36 plots were sampled, including 32 plots in the full N × P × K factorial design and the four plots receiving dolomite and micronutrients.

Soils were sampled to 10 cm depth using a 2.5 cm diameter corer at nine systematically distributed points in the center 20 × 20 m quadrat of each plot. Samples from each plot were composited in the field and returned immediately to the laboratory, where roots, stones, and mesofauna were removed by hand. Soils were then stored overnight at 4°C before extraction for extractable nutrients and measurement of pH the following morning. A subsequent study (Turner and Romero, 2009) showed that overnight storage at 4°C can increase ammonium and nitrate concentrations by 1–4 mg N kg<sup>-1</sup> (depending on the soil), so results for extractable N should be interpreted in that context. In particular, later analyses of control plots with extraction of N the same day as sampling gave NH<sub>4</sub> and NO<sub>3</sub> concentrations of ~1 mg N kg<sup>-1</sup>.



**Fig. 1.** Daily rainfall on Barro Colorado Island (A) and soil water content in experimental plots on Gigante Peninsula (B), between October 2006 and December 2007. The dates of fertilizer addition and sampling, and the approximate extent of the wet season are shown. Error bars represent one standard error for four replicate plots in each treatment.

## Analytical

All analyses were performed on field-moist soils following overnight refrigeration. Moisture content was determined by drying for 24 h at 105°C. Soil pH was determined in a 1:2 soil to solution ratio in both deionized water and 10 mM CaCl<sub>2</sub>. Inorganic N was extracted in 0.5 M K<sub>2</sub>SO<sub>4</sub>, with ammonium and nitrate determined by automated colorimetry on a Lachat Quikchem 8500 (Hach Ltd, Loveland, CO). Extractable phosphate (i.e., exchangeable phosphate in competitive equilibrium with the soil exchange complex) was extracted by anion exchange membranes (AEM-P), elution of the phosphate in 0.25 M H<sub>2</sub>SO<sub>4</sub>, and detection by automated molybdate colorimetry (Turner and Romero, 2009). Exchangeable cations and micronutrients were extracted in Mehlich-III solution (Mehlich, 1984), with determination of Al, Ca, Cu, Fe, K, Mg, Mn, and Zn by inductively-coupled plasma optical-emission spectrometry on an Optima 2100 (PerkinElmer, Inc, Shelton, CT). Mehlich extracts were also analyzed by molybdate colorimetry to determine extractable inorganic phosphate.

## Statistical

We performed repeated measures analyses of variance (ANOVA) for each response variable to evaluate temporal variation. Between-subject (or between-plot) effects evaluate responses over the entire experiment. Within-subject (or within-plot) effects evaluate variation among months and interactions among treatments and month. Repeated measures ANOVA assumes compound symmetry of the variance–covariance matrix if there are more than two repeated measures. We therefore used the conservative Greenhouse-Geisser correction for violations of the compound symmetry assumption. All analyses were performed with SYSTAT 11.0 (Richmond, CA).

The NPK factorial design is replicated four times along a 36-m north–south topographic gradient and blocked within each replicate along the perpendicular east–west gradient to control spatial variation in soil properties (Wright et al., 2011). However, the NP design does not account for blocks within replicates, so must overcome additional uncontrolled spatial variation. The quarterly NPK design therefore has greater power to detect treatment effects, while the monthly NP design has greater power to detect seasonal effects. Throughout the results we report treatment effects and seasonal K effects with respect to the quarterly NPK factorial design, and seasonal N and P effects with respect to the monthly NP factorial design. Time (month or quarter) × nutrient interactions allow us to isolate natural seasonal variation in the absence of nutrient addition from the influence of nutrient addition during the wet season. Time × nutrient interactions were evaluated in the monthly NP factorial design for N and P and in the quarterly NPK factorial design for K. Micronutrient treatment effects are reported as a direct contrast between control and micronutrient plots.

## RESULTS

### Climate during the Study Period

The dry season began at the end of December 2006 and continued until late April 2007, although there were a few days of heavy rain in early April (Fig. 1A). The maximum daily rainfall was 147 mm in November 2006. Soil moisture was highest (~0.8 g H<sub>2</sub>O g<sup>-1</sup> soil) in November 2006, when sampling occurred immediately following the highest rainfall event, but was otherwise relatively stable throughout the wet season (0.60–0.75 g H<sub>2</sub>O g<sup>-1</sup> soil) (Fig. 1B). Dry season soil moisture was lowest in March ( $F_{12,108} = 103.0, p < 0.001$ ), when values ranged between 0.34 and 0.40 g H<sub>2</sub>O g<sup>-1</sup> soil. There was significantly greater moisture under N addition in the quarterly NPK design ( $F_{1,18} = 5.8, p = 0.026$ ; Table 1), but no significant variation among treatments in the monthly NP factorial design ( $p > 0.10$ ).

### Soil pH

Nitrogen addition acidified the soil significantly for measurements made in both deionized water and 10 mM CaCl<sub>2</sub> (e.g., for pH in water,  $F_{1,18} = 37.7, p < 0.001$ ; Fig. 2A). In contrast, P addition caused a modest increase in soil pH (e.g., for water,  $F_{1,18} = 5.1, p = 0.037$ ; Fig. 2A). The decline in pH with N addition tended to be greater with N alone ( $5.25 \pm 0.10$  in controls versus  $4.47 \pm 0.01$  in plots receiving N alone) compared to plots with added P ( $5.36 \pm 0.10$  in P addition plots versus  $4.85 \pm 0.11$  in plots receiving both N and P); however, this N × P interaction was insignificant ( $F_{1,18} = 2.0, p = 0.18$ ).

There was significant seasonal variation in soil pH in water ( $F_{12,108} = 19.6, p < 0.001$ ), with lower values in the dry season than the wet season (Fig. 2B). Similar treatment and seasonal effects were observed for measurements in CaCl<sub>2</sub> (Table 1), although pH values were approximately half a pH unit lower in CaCl<sub>2</sub> than in water. Thus, values in CaCl<sub>2</sub> in the quarterly NPK design were  $4.77 \pm 0.11$  for control plots compared to  $4.04 \pm 0.03$  in plots receiving N alone (data not shown).

### Extractable Ammonium and Nitrate

Ammonium concentrations were not affected by N addition ( $p > 0.10$ ; Fig. 3A). A significant N × P interaction in the quarterly NPK design ( $F_{1,18} = 7.0, p = 0.016$ ) arises because ammonium was always lowest where N and P were added together (not shown). The mean ammonium concentration between November 2006 and November 2007 (inclusive) in the quarterly NPK design was  $4.84 \pm 0.36$  mg N kg<sup>-1</sup> in plots receiving neither N nor P,  $6.35 \pm 1.11$  mg N kg<sup>-1</sup> in plots receiving N but not P,  $5.39 \pm 0.71$  mg N kg<sup>-1</sup> in plots receiving P but not N, and  $2.81 \pm 0.35$  mg N kg<sup>-1</sup> in plots receiving both N and P (Fig. 3A).

Ammonium concentrations varied seasonally ( $F_{12,108} = 13.1, p < 0.001$ ); values were lowest in the dry season and highly variable in the wet season for all treatments (including control plots), with pulses in May and July following fertilizer application (Fig. 3B). There were no corresponding pulses in

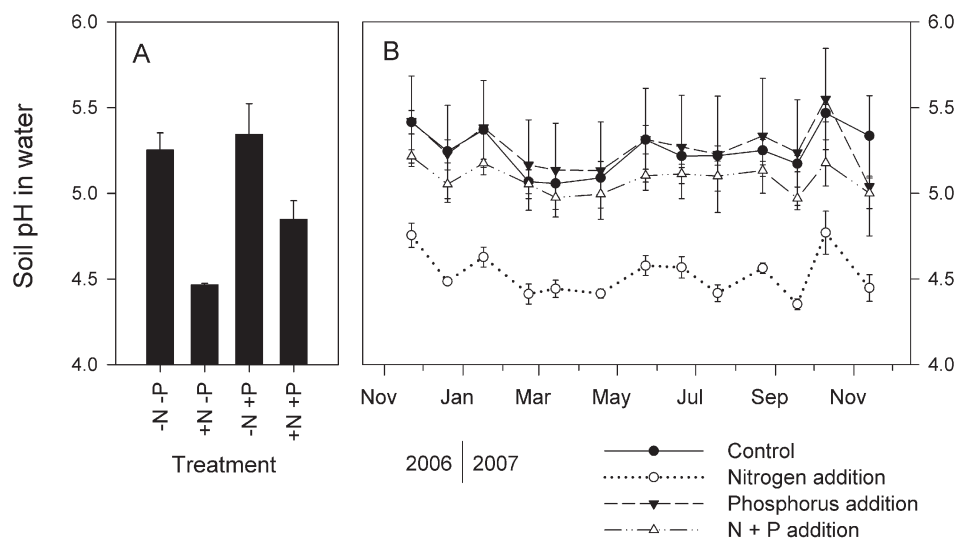
**Table 1. Summary of seasonal and treatment effects for soil moisture, pH, and extractable nutrients. Treatment effects were evaluated in the quarterly NPK factorial design, seasonal effects in the monthly NP factorial design, and time × nutrient interactions in the NP design except for extractable K, which was evaluated in the NPK factorial design. *F* values are shown in parentheses, with significant effects indicated by \*, \*\*, and \*\*\*, representing probability at the 5%, 1%, and 0.1% levels, respectively. AEM, anion exchange membrane; ns, not significant.**

Parameter	Treatment ( $F_{1,18}$ )	Season ( $F_{12,108}$ )	Time × nutrient interactions ( $F_{12,108}$ )
Soil moisture	Increased by N ( $F = 5.8^*$ )	Lowest in dry season ( $103.0^{***}$ )	ns
Soil pH (water)	Reduced by N ( $F = 37.7^{***}$ ) Increased by P ( $F = 5.1^*$ )	Lowest in dry season ( $F = 19.6^{***}$ )	ns
Soil pH (CaCl <sub>2</sub> )	Reduced by N ( $F = 32.4^{***}$ ) Increased by P ( $F = 4.6^*$ )	Lowest in dry season ( $F = 18.5^{***}$ )	ns
NH <sub>4</sub>	N × P interaction ( $F = 7.0^*$ )	Lowest in dry season and variable in wet season ( $F = 13.1^{***}$ )	Time × N interaction ( $F = 3.0^*$ ) Time × P interaction ( $F = 3.0^*$ )
NO <sub>3</sub>	Increased by N ( $F = 88.0^{***}$ ) Increased by P ( $F = 22.2^{***}$ ) N × P interaction ( $F = 12.9^{**}$ )	Very high in dry season ( $F = 16.8^{***}$ )	Time × N × P interaction ( $F = 5.9^{**}$ )
Mehlich P	Increased by P ( $F = 56.4^{***}$ )	Lowest in dry season ( $F = 3.8^*$ )	Time × P interaction ( $F = 3.7^*$ )
AEM P	Increased by P ( $F = 122.2^{***}$ ) Increased by K ( $F = 5.1^*$ ) P × K interaction ( $F = 4.8^*$ )	Lowest in dry season ( $F = 10.1^{***}$ )	Time × P interaction ( $F = 10.3^{***}$ )
Extractable Al	Increased by N ( $F = 16.0^{**}$ )	Variable ( $F = 21.8^{***}$ )	Time × N interaction ( $F = 2.3^*$ )
Extractable Ca	Reduced by N ( $F = 6.6^*$ )	Lowest in dry season ( $F = 13.3^{***}$ )	Time × N interaction ( $F = 2.4^{**}$ )
Extractable K	Reduced by N ( $F = 16.0^{**}$ ) Increased by K ( $F = 36.2^{***}$ ) N × K interaction ( $F = 5.8^*$ )	Highest in early wet season ( $F = 8.5^{***}$ )	Time × K interaction (NPK: $F = 6.3^{**}$ )
Extractable Mg	ns	Variable ( $F = 10.1^{***}$ )	ns
Extractable Cu	Increased by K ( $F = 9.0^{**}$ )	Lowest in dry season ( $F = 10.9^{***}$ )	ns
Extractable Fe	Increased by N ( $F = 9.1^{**}$ )	Lowest in dry season ( $F = 15.2^{***}$ )	ns
Extractable Mn	ns	Variable ( $F = 14.1^{***}$ )	ns
Extractable Zn	ns	Lowest in dry season ( $F = 10.1^{***}$ )	ns

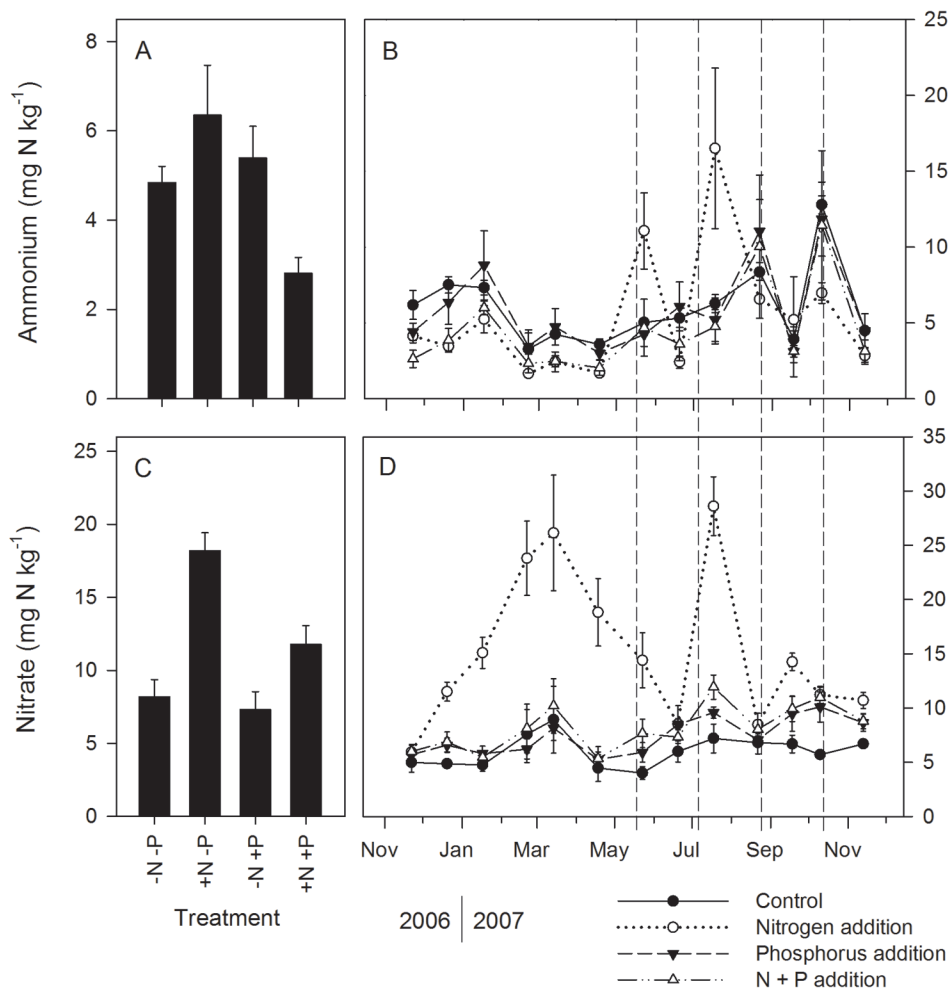
August and October, presumably because sampling occurred just before fertilization (compare Fig. 1 and Fig. 3). There were significant time × N and time × P interactions (in both cases  $F_{12,108} = 3.0, p < 0.05$ ).

Nitrate concentrations were increased by N addition ( $F_{1,18} = 88.0, p < 0.001$ ; Fig. 3C). The increase was muted strongly by P addition, as indicated by the significant N × P interaction ( $F_{1,18} = 12.9, p = 0.002$ ; Fig. 3C). Nitrate concentrations varied seasonally ( $F_{12,108} = 16.8, p < 0.001$ ), with a marked increase in the dry season, particularly in the N only treatment, although this was reduced markedly by P addition (Fig. 3D). There was a significant time × N × P interaction ( $F_{12,108} = 5.9, p = 0.004$ ; Table 1). A very high nitrate concentration in July 2007 occurred shortly after fertilization. Mean nitrate concentrations between November 2006 and November 2007 (inclusive) in the quarterly NPK factorial design were  $8.19 \pm 1.18 \text{ mg N kg}^{-1}$  in

plots receiving neither N nor P,  $18.2 \pm 1.22 \text{ mg N kg}^{-1}$  in plots receiving N but not P,  $7.32 \pm 1.22 \text{ mg N kg}^{-1}$  in plots receiving P but not N, and  $11.8 \pm 1.3 \text{ mg N kg}^{-1}$  in N plus P plots. This equates to an approximate doubling of nitrate in plots receiving N but not P.



**Fig. 2. Soil pH measured in deionized water in fertilized plots on Gigante Peninsula, Panama, between November 2006 and November 2007, showing (A) mean values for the quarterly NPK factorial design, with the significant treatment effects for N and P addition, and (B) seasonal variation in the monthly NP factorial design, showing the significant effect of N addition. Error bars represent one standard error for eight plots (A) or four plots (B) in each treatment. Statistical details are given in Table 1.**



**Fig. 3. Concentrations of extractable ammonium (A, B) and nitrate (C, D) in fertilized plots on Gigante Peninsula, Panama, between November 2006 and November 2007. The effect of treatments is shown in the left panels (A, C) and seasonal changes are shown in the right panels (B, D). Fertilizer application is shown by dashed vertical lines, but actually occurred over a 3-d period around the date. Panel A shows the absence of a significant nitrogen or phosphorus effect on ammonium, but a significant  $N \times P$  interaction. Panel C shows the significant effects of both N and P addition on nitrate. Error bars represent one standard error for eight plots (A, C) or four plots (B, D) in each treatment. Statistical details are given in Table 1.**

### Extractable Phosphate

We measured extractable phosphate by two methods: by extraction with anion exchange membranes (AEM-P) and by Mehlich-III solution (Mehlich P). In both cases, concentrations in control plots were extremely low ( $\leq 1 \text{ mg P kg}^{-1}$ ).

Phosphorus addition increased Mehlich P tremendously ( $F_{1,18} = 56.4, p < 0.001$ ; Fig. 4A). Across the annual cycle in the quarterly NPK design, the mean Mehlich P concentration in no-P plots was  $0.57 \pm 0.02 \text{ mg P kg}^{-1}$ , compared to  $16.72 \pm 1.87 \text{ mg P kg}^{-1}$  in P addition plots (Fig. 4A), a 29-fold increase. Mehlich P concentrations varied seasonally ( $F_{12,108} = 3.8, p = 0.018$ ), with a significant time  $\times$  P interaction ( $F_{12,108} = 3.7, p = 0.022$ ). This occurred because Mehlich P was lowest in March in plots receiving P, but there was no clear trend in no-P plots (Fig. 4B).

Anion-exchange membrane P was increased tremendously by the addition of P ( $F_{1,18} = 122.2, p < 0.001$ ; Fig. 4C). For AEM-P there was a further increase with K addition in plots that also received P, as indicated by the significant  $P \times K$  interaction ( $F_{1,18}$

$= 4.8, p = 0.041$ ; Fig. 4C). The mean AEM-P concentration across the annual cycle in the quarterly NPK design was  $0.71 \pm 0.10 \text{ mg P kg}^{-1}$  in plots receiving neither P nor K,  $32.2 \pm 3.8 \text{ mg P kg}^{-1}$  in plots receiving P but not K,  $0.90 \pm 0.10 \text{ mg P kg}^{-1}$  in plots receiving K but not P, and  $48.1 \pm 6.3 \text{ mg P kg}^{-1}$  in plots receiving both P and K (Fig. 4C). The effect of K addition on Mehlich-III P was marginally insignificant ( $p = 0.06$ ; not shown). There was strong seasonal variation in AEM-P ( $F_{12,108} = 10.1, p < 0.001$ ) and a significant time  $\times$  P interaction ( $F_{12,108} = 10.3, p < 0.001$ ), because the lowest values in P addition plots occurred in the dry season, presumably reflecting the onset of P fertilization in May. There was no clear seasonal trend in no-P plots (Fig. 4B).

### Extractable Aluminum and Base Cations

Extractable Al was increased by N addition ( $F_{1,18} = 16.0, p = 0.001$ ; Fig. 5A). Mean extractable Al concentrations were  $1154 \pm 41 \text{ mg Al kg}^{-1}$  in no N plots and  $1365 \pm 34 \text{ mg Al kg}^{-1}$  in N addition plots, an 18% increase. Extractable Al varied seasonally ( $F_{12,108} = 21.8, p < 0.001$ ), although values were variable

and there was no clear trend (Fig. 5B). A significant time  $\times$  N interaction ( $F_{12,108} = 2.3, p = 0.013$ ) appears to be due to the increase in extractable Al with N addition being greater in the late wet season than the rest of the year (Fig. 5B).

Extractable Ca was reduced by N addition ( $F_{1,18} = 6.6, p = 0.02$ ). Mean concentrations in the NPK design were  $1707 \pm 136 \text{ mg Ca kg}^{-1}$  in no-N plots and  $1276 \pm 114 \text{ mg Ca kg}^{-1}$  in N addition plots, a 25% decline (Fig. 5C). The reduction in Ca with N addition appears to be ameliorated where P is also added, presumably because triple superphosphate contains 15% Ca, but the  $N \times P$  interaction was not significant (data not shown). Extractable Ca varied seasonally ( $F_{12,108} = 13.3, p < 0.001$ ), with lowest concentrations in the dry season (Fig. 5D). A significant time  $\times$  N interaction ( $F_{12,108} = 2.4, p = 0.008$ ) arises because the decline in Ca with N addition was greater in the late wet season and early dry season. Extractable Ca was not affected by the micronutrient treatment ( $p > 0.10$ ; data not shown), despite addition of dolomite.

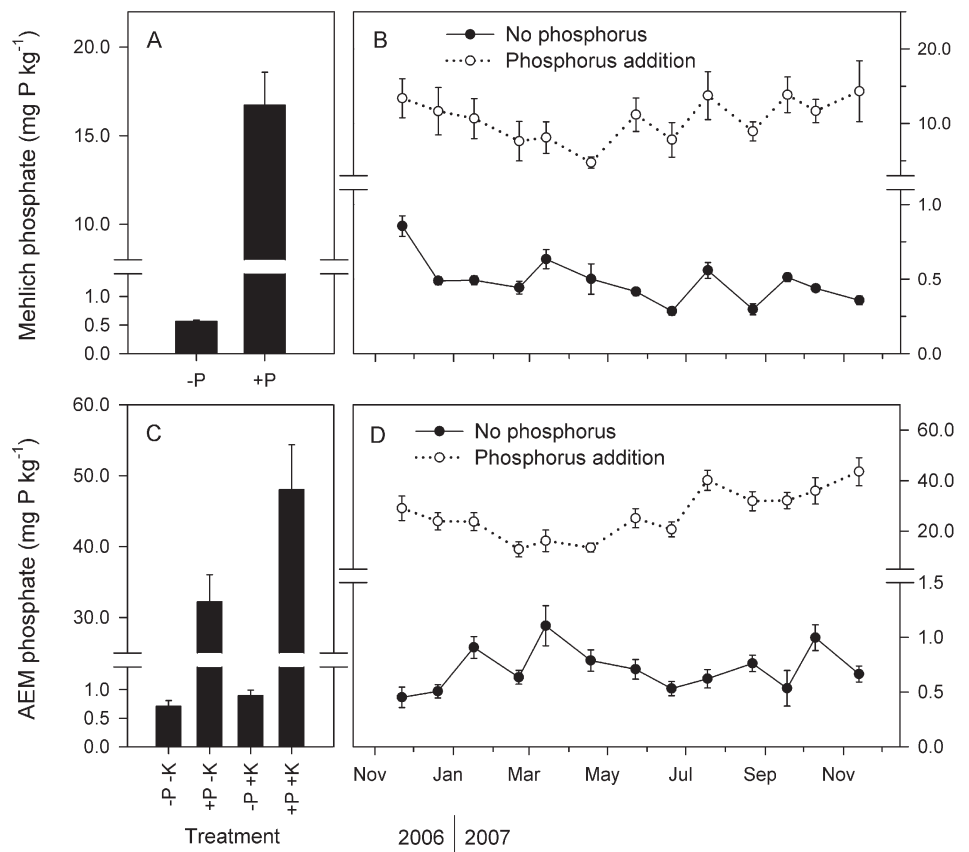
Extractable Mg did not vary significantly among treatments ( $p > 0.10$ ) despite a lower concentration in N addition plots (Fig. 5E), but varied seasonally ( $F_{12,108} = 10.1, p < 0.001$ ; Fig. 5F). Mean concentrations in the quarterly NPK design were  $486 \pm 37 \text{ mg Mg kg}^{-1}$  in no-N plots and  $401 \pm 47 \text{ mg Mg kg}^{-1}$  in N addition plots, a 17% decline (Fig. 5E). Extractable Mg was not affected by the micronutrient treatment ( $p > 0.10$ ; data not shown), despite addition of dolomite.

Extractable K was increased by K addition ( $F_{1,18} = 36.2, p < 0.001$ ) and reduced by N addition ( $F_{1,18} = 16.0, p = 0.001$ ; Fig. 5G). A significant  $N \times K$  interaction ( $F_{1,18} = 5.8, p = 0.027$ ) reflected a small reduction in extractable K between the control and N addition treatments and a much larger reduction between the K addition and N plus K addition treatments (Fig. 5G). Mean concentrations in the quarterly NPK design were  $103 \pm 20 \text{ mg K kg}^{-1}$  in plots receiving neither N nor K,  $197 \pm 26 \text{ mg K kg}^{-1}$  in K addition plots,  $83 \pm 17 \text{ mg K kg}^{-1}$  in N addition plots, and  $129 \pm 12 \text{ mg K kg}^{-1}$  in K plus N plots (Fig. 5G). These values corresponded to a 91% increase in plots receiving K but not N, and a modest 25% increase for plots receiving both K and N. The decline in extractable K in plots receiving N compared to those receiving no N was 19%. Extractable K varied seasonally ( $F_{12,108} = 8.5, p < 0.001$ ), with concentrations being highest in the early part of the wet season (Fig. 5H). A significant  $\text{time} \times K$  interaction ( $F_{12,108} = 6.3, p = 0.001$ ) in the quarterly NPK factorial design occurred because the increase in extractable K with K addition was greater in the wet season than the dry season.

### Extractable Micronutrients

Extractable Cu was increased with K addition ( $F_{1,18} = 9.0, p = 0.008$ ), but not with P or N ( $p > 0.10$ ). The mean Cu concentration was  $2.77 \pm 0.17 \text{ mg Cu kg}^{-1}$  in no-K plots and  $3.29 \pm 0.23 \text{ mg Cu kg}^{-1}$  in plots receiving K, an increase of 19% (Fig. 6A). The separate micronutrient treatment increased extractable Cu to  $5.25 \pm 0.63 \text{ mg Cu kg}^{-1}$  (Fig. 6A), a 90% increase compared to the no-K controls ( $F_{1,6} = 10.6, p = 0.017$ ). Extractable Cu varied seasonally ( $F_{12,108} = 10.9, p < 0.001$ ), with lowest concentrations in the dry season and the mid-wet season (Fig. 6B).

Extractable Fe was increased with N addition ( $F_{1,18} = 9.1, p = 0.007$ ). The mean Fe concentration was  $203 \pm 4 \text{ mg Fe kg}^{-1}$  in

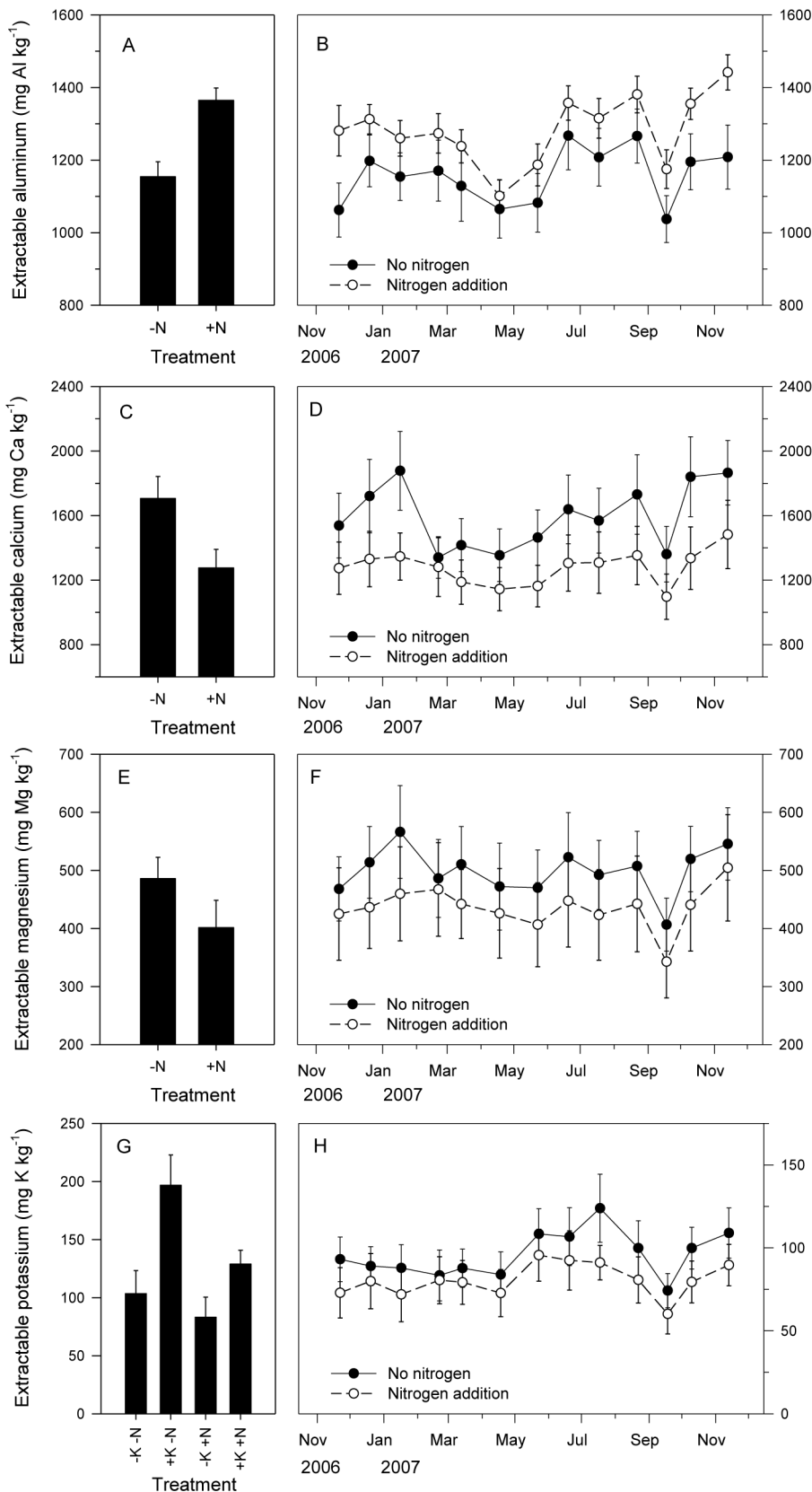


**Fig. 4. Concentrations of extractable inorganic phosphate determined by extraction in Mehlich-III solution (A, B) and with anion exchange membranes (C, D) in fertilized plots on Gigante Peninsula, Panama, between November 2006 and November 2007. The significant effect of P addition is shown in all panels, while panel C also shows the significant effect of potassium addition (with a  $P \times K$  interaction). Error bars represent one standard error for 16 plots (A) or 8 plots (B,C,D) in each treatment. Statistical details are given in Table 1.**

no-N plots and  $224 \pm 7 \text{ mg Fe kg}^{-1}$  in plots receiving N, an increase of 10% (Fig. 6C). The separate micronutrient treatment decreased extractable Fe to  $163 \pm 28 \text{ mg Fe kg}^{-1}$  ( $F_{1,6} = 7.6, p = 0.033$ ), a 20% decrease compared to the no-N controls (Fig. 6C). Extractable Fe varied seasonally ( $F_{12,108} = 15.2, p < 0.001$ ), with lowest concentrations in the dry season and the mid-wet season (Fig. 6D).

Extractable Mn was not affected by nutrient addition ( $p > 0.10$ ). The mean concentration for all plots in the quarterly NPK design was  $395 \pm 24 \text{ mg Mn kg}^{-1}$ . The separate micronutrient treatment did not affect extractable Mn ( $p > 0.10$ ); the mean concentration in the micronutrient plots was  $411 \pm 133 \text{ mg Mn kg}^{-1}$  (Fig. 6E). There was significant seasonal variation in extractable Mn ( $F_{12,108} = 14.1, p < 0.001$ ), with lowest concentrations in the dry season and mid-wet season (Fig. 6F).

Extractable Zn was not affected by nutrient addition ( $p > 0.10$ ). The mean concentration for all plots in the quarterly NPK design was  $3.78 \pm 0.37 \text{ mg Zn kg}^{-1}$ . The separate micronutrient treatment increased extractable Zn significantly to  $7.76 \pm 1.37 \text{ mg Zn kg}^{-1}$  ( $F_{1,6} = 8.4, p = 0.028$ ), an increase of 105% (Fig. 6G). There was significant seasonal variation ( $F_{12,108} = 10.1, p < 0.001$ ), with lowest concentrations in the dry season and mid-wet season (Fig. 6H).



**Fig. 5.** Concentrations of extractable aluminum (A, B) and the base cations Ca (C, D), Mg (E, F), and K (G, H) in fertilized plots on Gigante Peninsula, Panama, between November 2006 and November 2007. The significant effect of N addition in the NPK factorial design are shown in A, C, and G (but note that the effect is not significant in panel E), while panel G also shows the significant K addition (with a N  $\times$  K interaction). Panels B, D, F, and H show the significant seasonal variation in the NP factorial design. Error bars represent one standard error for 16 plots (A,C, E) or 8 plots (all other panels) in each treatment. Statistical details are given in Table 1.

## DISCUSSION

### Seasonal Effects on Extractable Soil Nutrients

Soil nutrient concentrations are expected to vary during the annual cycle in seasonal tropical forests because rainfall at the onset of the wet season should release a pulse of nutrients from litter decomposition and lysis of microbial cells (Cornejo et al., 1994; Lodge et al., 1994; Wieder and Wright, 1995; McGrath et al., 2000). We predicted that this would be greatest for mobile nutrients such as N and K that are weakly retained in the soil, and least for nutrients such as P for which extractable concentrations are biologically and chemically buffered by the much larger stable inorganic and organic pools in the soil. Although we found significant temporal variation in a number of soil properties, only extractable K followed the expected pattern of higher concentrations in the early wet season. This is presumably due to rapid leaching of K from litter (Attiwill, 1968; Schreeg et al., 2013b) compared to the slower release of less soluble nutrients like Ca that occur in structural parts of the leaves and are released more slowly throughout the wet season (Cornejo et al., 1994; Yavitt and Wright, 1996). It is likely that our monthly sampling regime missed short-term nutrient pulses, although the absence of a broad increase in nutrient availability at the onset of the wet season provides some support for the suggestion that nutrient pulses are more common in dry tropical forests with a 6-mo dry season (Singh et al., 1989; Campo et al., 1998) than in semi-deciduous forests with a 4-mo dry season (Yavitt et al., 1993). This might occur because the length of the dry season influences the degree of deciduousness (and therefore the amount of litter fall in the dry season), as well as the degree of microbial desiccation and associated nutrient release at the end of the dry season (West et al., 1992).

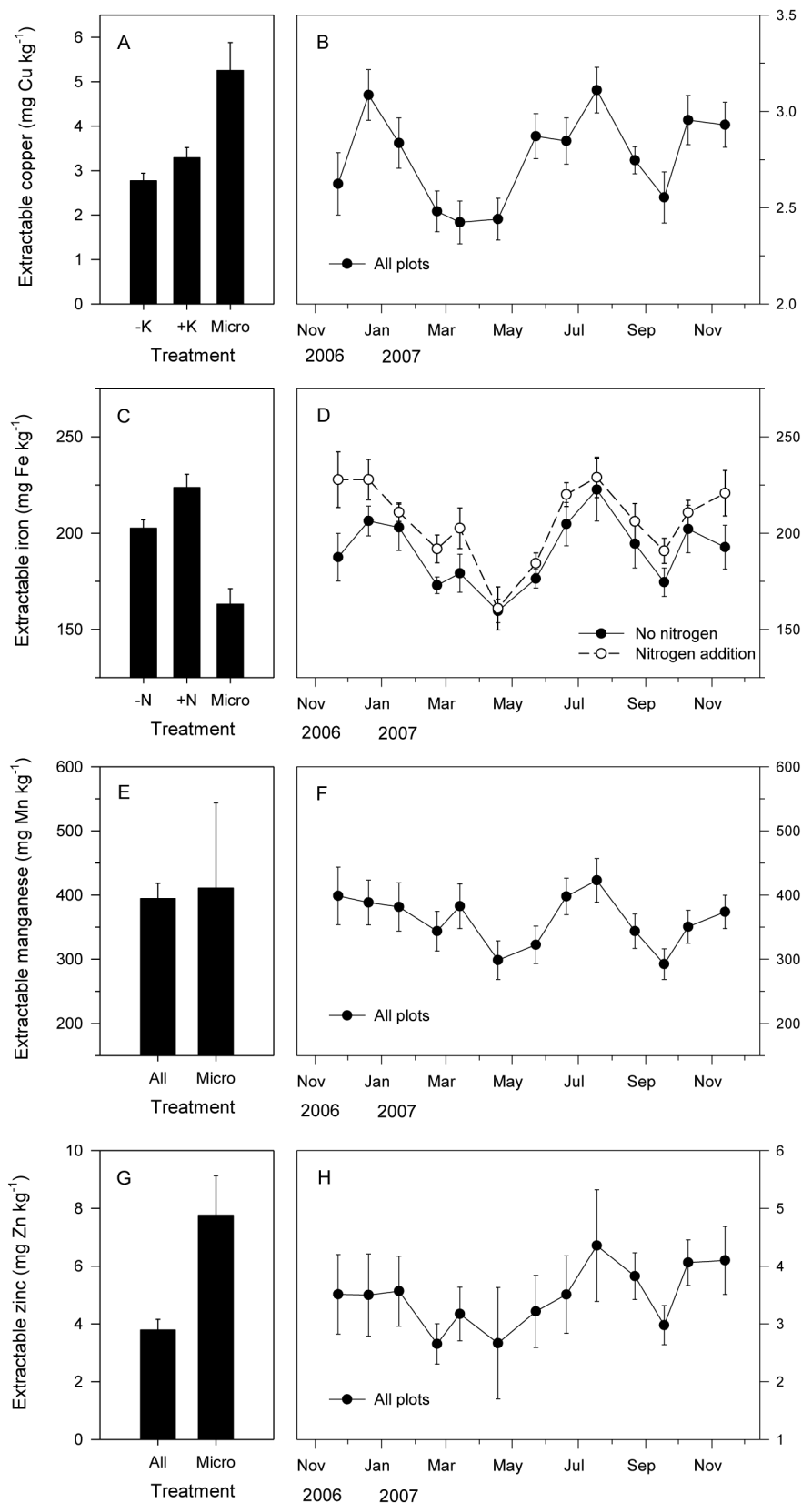
Of the other soil properties studied, soil pH and the concentrations of a number of extractable nutrients, including ammonium, Ca, Cu, Fe, P, and Zn were lower in the dry season than in the wet season. A number of factors might



contribute to this, including a reduction in litter decomposition and leaching in the dry season, reduced microbial activity and maintenance of nutrients in extractable forms, and chemical and physical changes linked to soil drying (including changes in pH). For example, soil drying ruptures organic coatings on mineral particles (Bartlett and James, 1980), increases the surface area for sorption reactions (Haynes and Swift, 1989), disrupts aggregates (Amézqueta, 1999), and influences the crystallization of metal oxides (McLaughlin et al., 1981), all of which can influence extractable nutrient concentrations.

In contrast, nitrate concentrations were highest in the dry season, particularly in plots receiving N but not P or K. This confirms a pattern reported previously (Koehler et al., 2009) and might indicate a combination of reduced nitrate leaching in the absence of rainfall between January and April and reduced denitrification in the more acidic N-only plots compared to the less acidic N plus P plots (denitrification is greatly reduced at pH < 4.8; Bremner and Shaw, 1958).

Fertilizer was added during the wet season in this study, so we used time × nutrient interactions to isolate true seasonal effects from those that might arise through wet-season fertilizer addition (Table 1). For example, fertilizer addition almost certainly accounted for pulses of ammonium during the wet season (Koehler et al., 2009), although wet-season pulses of ammonium in plots receiving N alone, but not in the N plus P treatment, might be linked to a reduction in ammonium oxidation in the more acidic soils of the N only plots (Paul and Clark, 1996). Similarly, P addition presumably accounted for the seasonal pattern in extractable phosphate. Extractable phosphate in P addition plots declined during the dry season, which is consistent with the effect of adding fertilizer throughout the wet season and its slow movement into more stable pools (Bramley et al., 1992), perhaps in conjunction with an increase in phosphate sorption capacity following disruption of organic matter coatings on mineral surfaces as soils dry (Haynes and Swift, 1989). However, extractable phosphate concentrations in plots that did not receive P addition did not



**Fig. 6.** Concentrations of the extractable micronutrients Cu (A, B), Fe (C, D), Mn (E, F), and Zn (G, H) in fertilized plots on Gigante Peninsula, Panama, between November 2006 and November 2007. For Cu, the significant effect of K addition is shown in panel A, but the seasonal pattern is shown as the mean of all plots in the NP factorial design (panel B). The significant effect of N addition on iron is shown in panels C and D. There were significant effects of micronutrient addition for Cu (A) and Zn (G). Error bars represent one standard error for four plots (micronutrient treatments), eight plots (D), 32 plots (E,G) or 16 plots (all other panels) in each treatment. Statistical details are given in Table 1.

vary seasonally (or were slightly higher in the dry season). This is consistent with the buffering capacity of the soil and rapid microbial transformations maintaining a relatively constant extractable phosphate pool (Frossard et al., 1995; Obserson and Joner, 2005).

Trace metals such as Cu, Mn, and Zn are stabilized in soils via interactions with cation exchange sites on clays, metal oxides, and organic matter (Adriano, 1986), although there is variation among elements that might contribute to differences in seasonal patterns of extractability (Adriano, 1986). For example, in most soils Cu is considered relatively immobile and Zn relatively mobile, while Mn is influenced strongly by redox changes (Adriano, 1986). We detected significant seasonal variation in Cu, Mn, and Zn, with lower concentrations generally occurring in the dry season. This is most likely explained by effects of drying on hydrous metal oxides (McLaughlin et al., 1981), because these compounds have a considerable influence on the stability of trace metals (Jenne, 1968; Tack et al., 2006).

There are many examples of seasonal changes in soil nutrients in temperate and subarctic forests (e.g., Weih, 1998; Chen et al., 2003), but relatively few detailed studies of seasonal variation in soil nutrients in the lowland tropics. No seasonal variation was found in either inorganic N or Bray-1 P measured monthly over 5 yr in an irrigation experiment on Barro Colorado Island (Yavitt and Wright, 1996), a few km from the present study site. Indeed, there were few changes in soil nutrient status when the dry season was experimentally 'removed' by irrigation (Yavitt et al., 1993; Yavitt and Wright, 1996). In contrast, resin P concentrations were higher in the early wet season in an Ultisol in western Amazonian (McGrath et al., 2000), but were higher in samples taken in the dry season than in the wet season for an Oxisol in Costa Rica, although there was no difference in a nearby Mollisol (Cleveland et al., 2004). The apparent seasonal variation in inorganic N reported in some studies seems likely to be an artifact of soil storage before extraction, as indicated by extremely high inorganic N concentrations (Luizão et al., 1992; Cleveland et al., 2004).

### **Treatment Effects Following a Decade of Fertilizer Addition**

We predicted that the response of extractable nutrients to long-term fertilizer addition would vary among individual nutrients depending on the nature of their biogeochemical cycles. For N, we predicted that its transient nature would promote a modest increase in response to chronic fertilizer addition, and this appears to be the case. We found no treatment effects on ammonium concentrations, although nitrate concentrations were greater with N addition. This is similar to the effects reported after four (Yavitt et al., 2011), seven (Sayer et al., 2012), and 9 yr (Koehler et al., 2009) of fertilization, although concentrations of both ammonium and nitrate in these studies were larger than those reported here, presumably due to storage effects (Turner and Romero, 2009). No changes in N availability were reported for tropical montane forest soils in Hawaii following long-term

N fertilization, at least for sites where productivity was limited by P alone or in combination with N (Hall and Matson, 2003). However, greater nitrification rates were detected in that study, which appeared to arise through an increase in the activity of nitrifying organisms. This also appears to explain the overall increase in nitrate following long-term N addition detected here (Koehler et al., 2009; Corre et al., 2010).

For P, we expected extractable P concentrations to increase slowly but continuously as chronic P addition saturated sorption sites. Four years of P addition increased Bray-1 P by 50% (Yavitt et al., 2011) and this trend has continued, because after a decade of P addition we measured extractable P concentrations up to 50 times greater than in no-P plots. This indicates that chronic fertilizer addition is now overcoming the inherently high P sorption capacity of the soils (Schreeg et al., 2013a).

Potassium addition has had important effects on plant growth, leading to a marked reduction in fine root growth and, when applied with N, to a significant amelioration of the long-term decline in trunk growth rates (Wright et al., 2011; Yavitt et al., 2011). Potassium addition also influenced seedling performance, increasing relative height growth rate and reducing the root-to-shoot ratio of five species common to all plots, despite a modest 4% increase in tissue K after 2 yr of treatments (Santiago et al., 2012). We predicted a rapid but modest increase in extractable K in response to chronic K addition, based on the mobility of K during decomposition and its weak retention in soil. After 4 yr of fertilization, Yavitt et al. (2011) reported that extractable K had increased in K addition plots but decreased in N addition plots, and this pattern is confirmed by the data reported here. However, leaf litter K increased by 16% in both K addition plots and N addition plots during the first 6 yr of the experiment (Kaspari et al., 2008); in the N addition plots, this could have occurred through a temporary increase in K availability at the onset of soil acidification, even though the long-term effect of N addition on extractable soil K has been a strong decline along with other base cations (see below).

For micronutrients we expected moderate increases in response to micronutrient fertilizer addition, but differences among elements in response to variation in their solubility (e.g., Cu is stabilized more strongly than Zn in most soils) (Adriano, 1986). We found that Cu and Zn were increased to a similar extent (twofold) by the addition of micronutrient fertilizer, although extractable Mn was not increased significantly. This suggests that added Mn either leached rapidly following fertilizer addition or, more likely, entered unextractable Mn pools to a greater extent than Cu and Zn. The significant effect of K addition on extractable Cu is most likely due to the presence of Cu contamination in the K fertilizer, given no comparable effects on Mn or Zn.

An important effect of N addition was a reduction in soil pH, which complicates interpretation of responses to this treatment. Soil acidification was detected after only 4 yr of N addition (Yavitt et al., 2011), but does not appear to have strengthened after a further 6 yr of treatments. However,

acidification has reduced extractable cation concentrations in N addition plots, presumably by replacing cations with protons on cation exchange sites and through enhanced leaching. This decline was significant only for K after 4 yr (Yavitt et al., 2011), but after 10 yr was also significant for Ca (but not Mg), while concentrations of extractable Al and Fe increased in N addition plots. The decline in pH is therefore continuing to affect soil properties, even though pH did not change between 4 and 10 yr of N addition. Soil pH has a marked influence on trace metal concentrations, with increased solubility at pH < 5 (Adriano, 1986). However, there were no significant effects of N addition on extractable Cu, Mn, or Zn. Of significance is that the decline in extractable K concentrations in N addition plots offers insight into the significant N × K effect on stem diameter growth rates (Wright et al., 2011). Potassium addition returns extractable K to concentrations found in control soils, raising the possibility that the N × K effect on stem diameter growth rates reported previously is a true response to N addition.

Soil acidification by urea addition is well understood and also occurs to different extents with other N fertilizers (Bolan et al., 1991). A number of factors influence the decline in pH following urea application. Acidification is promoted by proton release during the oxidation of urea to nitrate and by the loss of nitrate by leaching. At the same time, acidification is reduced by the consumption of protons during denitrification and by the release of hydroxyl ions during nitrate uptake by plants (Bolan et al., 1991). The strongly-weathered nature of many tropical forest soils, including the soil on Gigante Peninsula, means they are more sensitive to N-induced acidification than less weathered soils that have a greater capacity to buffer the decline in pH through carbonate and cation exchange reactions (Matson et al., 2002). Although long-term N addition did not result in a significant pH decline in tropical montane forest in Hawaii (Hall and Matson, 2003; Lohse and Matson, 2005), the strong pH decline reported here is of a similar magnitude to changes found in agricultural studies. For example, Bouman et al. (1995) showed that for a temperate Mollisol of similar pH to the Oxisol studied here, an annual urea application at 180 kg N ha<sup>-1</sup> for 9 yr decreased soil pH from ~5.5 to 4.8. This was associated with a depletion of Ca and Mg by 11 and 18%, respectively, and a several-fold increase in soluble Al and Mn in soil solution. The effect of urea was less than for ammonia, while both were less acidifying than mono-ammonium phosphate or ammonium sulfate.

The decline in pH in the N treatment complicates interpretation of treatment effects, given the marked influence on a host of other soil chemical and biological properties. Aside from the decline in base cations and increase in soluble Al and Fe, considerable changes are expected to occur in microbial community composition and activity (Aciego Pietri and Brookes, 2008; Rousk et al., 2010) and enzyme activities (Turner, 2010). The likely increase in soluble Al following acidification, although not measured here directly (we measured extractable metals, which may not correspond to the solution concentrations), raises the possibility of Al toxicity in the N addition treatment.

Aluminum toxicity increases in soils at pH < 5.5 (Marschner, 2006) and it has been suggested that extractable Al influences the distribution of tree species on nearby Barro Colorado Island (Schreeg et al., 2010). There is little information on toxic Al concentrations for tropical trees, some of which may be adapted to withstand metal toxicity (e.g., members of the Rubiaceae and Vochysiaceae, which hyper-accumulate Al in their leaves; Jansen et al., 2002). However, the influence of Al toxicity on the productivity and distribution of tropical tree species warrants further attention, given the increasing rates of N deposition onto lowland tropical forests (Matson et al., 2002; Hietz et al., 2011).

## CONCLUSIONS

The response of extractable nutrients to seasonal climate and long-term fertilizer addition in a strongly weathered soil under lowland tropical forest varied among nutrients depending on the nature of their biogeochemical cycles. Extractable nutrients varied seasonally, with both decreases (e.g., cations and micronutrients) and increases (e.g., nitrate) in nutrient concentrations during the dry season. However, only extractable K appeared to increase during the early wet season, while extractable P varied little in no-P plots. A decade of P addition increased extractable P by 50-fold, while N addition approximately doubled nitrate concentrations but did not affect ammonium. The pH decline with N addition has stabilized at ~0.8 units lower than control soils, but continues to influence soil properties. In particular, base cation concentrations have declined markedly, while concentrations of the potential toxin Al have increased. The decline in extractable K in the N addition treatment, and its replenishment by K addition, suggests that the previously reported N × K interactive effect on trunk growth rates might be a true N effect. These results highlight the importance of the poorly-studied consequences of long-term atmospheric N deposition in regulating the productivity of lowland tropical forests.

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