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General introduction

1. Tropical secondary forests

1.1. Shifts in the tropical biome

Deforestation happens nowhere as fast as in the tropics. From 2000 to 2010 South America, Central America, Southeast Asia and Africa annually lost 0.45%, 1.19%, 0.23% and 0.49% of their forested area, respectively, combining for a net loss of 8.3 Mha forest per year, approximately the size of Austria (FAO, 2010). This reduction in tropical forest area corresponds to a net loss of approximately 1 Pg carbon (C) every year (Baccini et al., 2012). In fact, the gross rates of C emissions from tropical deforestation are about twice as high but are counterbalanced by carbon uptake from regenerating forests (Baccini et al., 2012; Pan et al., 2011). Thereby, on a global scale, deforestation is estimated to account for 6-17% of anthropogenic carbon emissions (van der Werf et al., 2009).
While tropical forests can be lost due to natural disasters such as volcanic eruptions, landslides or forest fires, the biggest contributor to deforestation and forest degradation is human activity. In particular, the conversion of forested area into agricultural land and pastures for livestock ranging drives the large-scale loss of tropical forests (Seymour & Harris, 2019). Commercial agriculture and small-scale subsistence agriculture contributed 40% and 33% to deforestation from 2000 to 2010, respectively. Urban expansion and forest-clearing for infrastructure projects each added another 10%.
Thinning of the canopy through logging, as well as other disturbances that do not lead to a complete forest clearing, are considered as forest degradation. Timber extraction accounted for 52% and fuelwood collection and charcoal production for 31% of forest degradation, while uncontrolled fires only caused 7% (Hosonuma et al., 2012).
Tropical forests have not only been experiencing fast rates of deforestation but also an ongoing increase of secondary forests (Malhi et al., 2014). Primary forests can be defined as forests that show no clear indication of human activities and remain largely undisturbed, therefore representing the local climax vegetation (FAO, 2012). Wherever

previously cleared land is abandoned or purposefully set aside for reforestation, the natural vegetation can regrow in a series of successional stages. These regenerating forests that usually differ from primary forests in structure and species composition are defined as secondary forests. The definition further extends to forests that have experienced significant degradation (FAO, 2012), and sometimes it's difficult to draw a clear line between the two forest categories. For example, selectively logged forests may be considered primary or secondary forests, depending on the rather subjective judgment of the intensity of disturbance (Chazdon, 2014; Chokkalingam & de Jong, 2001). In the same sense, old-growth secondary forests may be reclassified as primary forests, if the original species composition and processes have been largely re-established (FAO, 2012). In 2010 57% of the global forested area in the tropics was classified as secondary forest, whereas 36% of the area was in a primary state (FAO, 2010). Africa, Central America and Southeast Asia all reported more than 60% of their forested area as regenerating forest, whereas with about 75% of the total forest area South America still harbours the largest proportion of undisturbed forest. However, the losses of primary forest due to deforestation as well as reclassification as secondary forests are high. From 2000 to 2010 the annual primary forest loss rates ranged from 0.29% for Southeast Asia and 0.46% for South America to 1.12% for Africa and 1.52% for Central America (FAO, 2010). Given their sheer spatial extent and potential to partially re-establish the original function of primary forests if given enough time, it is fair to argue that secondary forests will likely play a prominent role in shaping the "tropical forest of the future" (Chazdon, 2014).

1.2. Potential for C sequestration by tropical secondary forests

Secondary forests can sequester large amounts of C in their regenerating biomass (Chazdon et al., 2016). During succession biomass build-up shows a typical pattern, with young forests exhibiting high growth rates that saturate with forest age as biomass levels approach that of primary forests (Poorter et al., 2016). In general, biomass recovery is fast. Secondary forests take on average 66 years to reach 90% of the biomass of their primary forest counterparts, albeit with huge variation between different sites (Poorter et al., 2016). For Latin America alone it has been estimated that if all currently

existing secondary forests up to 60 years in age were allowed to recover, between 6.7 and 10.9 Pg C could be sequestered in their biomass over the course of 40 years. Further, if 40% of the area used for agriculture was set aside for reforestation, an additional 2 Pg C could be sequestered (Chazdon et al., 2016). This approximately adds up to the current annual anthropogenic global net C emissions. However, it's uncertain to what extent the existing secondary forests will be allowed to regrow, as the future development of tropical land-use is hard to predict (Lewis et al., 2015). In addition, young secondary forests may primarily consist of forests used for slash-and-burn agriculture, and therefore only provide a transient C sink (Baccini et al., 2012; Neeff et al., 2006). Predicting that only 60% of the current secondary forests are allowed to regrow reduces the C sequestration potential to about 6 Pg C (Chazdon et al., 2016). However, the given values don't consider changes in belowground carbon storage in roots and soil organic carbon, and further exclude reforestation potential in tropical Africa and Southeast-Asia. Overall, this illustrates that the C sequestration potential in regenerating tropical forests is high, but that the magnitude of this effect will largely depend on the spatial extent of tropical forest regrowth.

1.3. Biodiversity conservation in tropical secondary forests

The highest eukaryotic diversity on earth is found in tropical ecosystems, yet due to habitat loss many species of mammals, birds and plants face their highest extinction threat in the tropics (Dirzo & Raven, 2003). Secondary forests could provide habitat for wildlife but their actual value for biodiversity conservationIs under debate. On average, secondary forests can reach similar tree species richness levels as primary forests after only 50 years of regrowth, but this can take much longer for vascular epiphytes (Martin et al., 2013; Rozendaal et al., 2019) Additionally, they usually contain only low proportions of primary forest species, and species composition may take centuries to recover (Rozendaal et al., 2019). Furthermore, individual studies showed diverging results. While some studies showed fast recovery of species richness (Dent & Wright, 2009; Gilroy et al., 2014; Lennox et al., 2018; Van Breugel et al., 2013), others didn't (Barlow et al., 2007; Gibson et al., 2011). In general, the extent to which secondary forests can restore biodiversity levels and species composition of primary forests seems

to depend largely on the landscape context they are found in. Dispersal limitation affects community assembly in all stages of succession, so that within a given landscape two forest patches resemble each other more the closer they are to each other (van Breugel et al., 2019). Therefore, the proximity to primary forests which can act as source pool of biodiversity is suggested to be important for repopulation success (Dent & Wright, 2009). Many studies that reported high levels of biodiversity recovery were conducted in areas with stretches of natural forest in close distance (Gilroy et al., 2014; Lennox et al., 2018; Van Breugel et al., 2013). Secondary forests recovering in landscapes with little remaining tree cover will likely show lower rates of biodiversity recovery.
Likewise, the degree of diversity recovery is highly dependent on time since start of secondary succession. During succession species richness is closely linked to biomass recovery and builds up over time (Gilroy et al., 2014), and species composition changes with succession. Fast-growing pioneer species dominate in early stages and are gradually replaced by slow-growing shade-tolerant species. Therefore, ephemeral secondary forests below 30 years of regrowth like the ones found in fallow-cycles provide little conservation value, since many of the slow-growing late-successional plants never reach the reproductive stage (Van Breugel et al., 2013). In conclusion, even if secondary forests are allowed to recover, their conservational value will largely depend on the remaining primary forest cover.

1.4. Successional effects on local climate and hydrology

On top of its role in C sequestration and biodiversity conservation, forest regrowth can have strong impacts on local climate and hydrology (Locatelli et al., 2015). Deforestation can affect climate not only via increased C release but also via biophysical effects. For example, forest loss in the boreal biome is actually expected to have an overall cooling effect on the climate due to an increased albedo effect caused by a lighter earth surface after tree removal (Anderson et al., 2011; Bala et al., 2007). In the tropics, however, deforestation is predicted to lead to net warming. This is mainly explained by a decrease in evapotranspiration from trees, leading to reduced evaporative cooling as well as reduced formation of clouds that shield the land surface from incoming solar radiation (Anderson et al., 2011; Bala et al., 2007). At the same time the reduction in tree

transpiration is predicted to decrease local precipitation (Lawrence & Vandecar, 2015). Deforestation can also affect the tropical water cycle by changes in soil hydrology. The "sponge effect" has been suggested as one mechanism of soil hydrological changes. It states that soils under forested areas have a higher water infiltration rate and capacity compared to the more compacted soils under degraded forests (Bruijnzeel, 2004), and therefore, deforestation usually increases water runoff rates (Evaristo & McDonnell, 2019). In a paired catchment experiment forested sites have been shown to have a balancing effect on the water cycle compared to pastures. While forest soils showed higher water runoff rates during the dry season, their total runoff rates during the wet season and peak runoff rates during rain events were lower compared to pasture soils (Ogden et al., 2013). With this effect on water runoff and evapotranspiration, forest cover can reduce flood risks during extreme weather events and help stabilize slopes against shallow soil erosion and landslides (Bradshaw et al., 2007; Robledo et al., 2004). All in all, tropical deforestation can cause local climate warming and aggravate flood severity. Therefore, reforestation might not only provide ecological but also direct socio-economic benefits.

2. Factors influencing tropical forest regrowth

As discussed in Chapter 1, tropical forests provide important ecosystem services that are increasingly taken over by secondary forests. The re-establishment of ecosystem functions and the extent to which secondary forests can restore ecosystem services thereby depend primarily on the level of biomass regrowth (Lohbeck et al., 2015). This brings up the question which factors are driving biomass accrual rates and the overall magnitude of regrowth.

2.1. Climate

Accurately predicting forest regrowth is an almost impossible task. On a small scale stochastic events like windfall, lightning or other disturbance events can affect succession in a way that even adjacent sites with the same land-use history and time of recovery can show diverging successional trajectories (Norden et al., 2015). When

looking for large-scale patterns, climate appears to be the strongest driver of biomass accrual rates in secondary forests. Tropical tree growth rates have been shown to increase with shorter dry season length and higher mean annual precipitation (Toledo et al., 2011), although this effect seems to mainly reflect the transition from dry tropical forests to more productive wet tropical forests, and the effect diminishes above a certain rainfall threshold. Stegen et al. (2011) found a relation between mean annual precipitation (MAP) and aboveground biomass only for tropical dry forests (MAP of 250-2000 mm according to the Holdridge life-zone classification; Holdridge (1947)), but not within moist or wet tropical forests (MAP of 2000-4000 mm or >4000 mm, respectively), with the highest biomass found in the wet tropics. Similarly, absolute growth rates of secondary tropical dry forests increase with MAP (Becknell et al., 2012), but this effect levels off for forests in areas with MAP above 2000 mm (Poorter et al., 2016), although this dataset didn't include areas with MAP above 4000 mm. Given the problem of comparing absolute growth rates, relative recovery rates (expressed as percentage of corresponding old-growth biomass) may be a more informative measure for a forests' ability to recover to original biomass levels, and indeed, relative biomass recovery rates were only very weakly related to MAP (Poorter et al., 2016). In conclusion, absolute growth rates, but not relative recovery rates, are higher in moist and wet tropical forests compared to dry forests. However, even within a given climatic region absolute growth rates can vary significantly and may be affected by differences in site management history or soil fertility.

2.2. Land-use history and soil fertility

Secondary forests can have a variety of different land-use histories, including differences in type of land-use (e.g. pasture versus agriculture), management practices (e.g. fertilization, shifting cultivation with or without burning of vegetation), duration of land-use and number of previous fallow cycles. These factors can affect secondary succession by generating a range of different soil starting conditions for forest regrowth (Powers & Marín-Spiotta, 2017). For example, in a tropical dry area in Mexico the number of previous fallow cycles explained reduced tree biomass in secondary forests (Eaton & Lawrence, 2009). Aboveground biomass C was significantly lower in plots that had been

cleared 3 or 4 times than in plots that had been cleared 1 or 2 times. In a secondary forest study in the Brazilian Amazon a management intensity index was used to predict the resilience of 5-year-old secondary forests recovering from shifting manioc agriculture (Jakovac et al., 2015). The index was calculated from the number of previous fallow cycles, the length of the most recent fallow period and the weeding intensity during manioc cropping. Management intensity decreased stand basal area and canopy height and increased the percentage of resprouting plants of the resulting secondary forests. The higher contribution of resprouters suggests that management history can affect the soil seed bank and thus vegetation structure of secondary forests, thereby potentially changing the trajectory of succession.

Additionally, management history can affect regrowth by altering soil fertility. Large tracts of the lowland tropics occur on old soils, where long-lasting soil weathering has led to an ongoing depletion of available phosphorus (P) and base cations relative to nitrogen (N), which enters ecosystems mainly through biological N fixation (Vitousek et al., 2010; Walker & Syers, 1976; Yang & Post, 2011). Therefore, tropical forests are usually considered as being primarily limited by P, and recent empirical evidence shows that P limitation of individual tree species is widespread throughout lowland tropical forests (Turner et al., 2018). Management history can potentially affect the magnitude of nutrient limitation as well as shift the identity of the limiting nutrients, since forest clearing, especially in slash-and-burn agriculture, can lead to significant losses of nutrients, with losses of N exceeding those of P (Davidson & Martinelli, 2009; Kauffman et al., 2009). A modelling study recently predicted that management practices that lead to the removal of biomass from cleared forest sites slows down forest regrowth by nutrient losses from the system and that these nutrient losses can lead to intermittent N limitation of early secondary forests (Nagy et al., 2017). In line with these predictions, Zarin et al. (2005) used data on burning history and aboveground biomass of 90 secondary forest plots in the Brazilian Amazon to show that biomass C accumulation in sites that had experienced 5 or more cycles of burning can be almost 50% lower than what models predict for these sites.

2.3. Evidence for nutrient limitation of forest regrowth

In a summary of different fertilization experiments across the lowland tropical rainforest region, Wright et al. (2018) found that secondary forests responded stronger to fertilization than primary forests, with no difference in their response to P and N addition. They concluded that the rapidly regenerating aboveground biomass of secondary forests could provide a stronger nutrient sink and thus a higher potential for nutrients to limit biomass build-up. Individual fertilization studies showed inconsistent trends. Markewitz et al. (2012) found no difference in biomass increment 6 years after P addition to a 24-year-old secondary forest. However, they only included 3 replicate plots and individual tree growth rates could only be compared for 3 species. In contrast, in a tropical dry forest in Mexico one out of three tree species responded to N, P and N+P fertilization in 10-year-old forest plots, whereas in the 60-year-old secondary forest two out of three responded to P and N+P addition only (Campo et al., 2012). Similarly, in a study in the Brazilian Amazon, where freshly-cleared forest patches were factorially fertilized with N, P and a mixture of micronutrients, responses to fertilization were highly species-specific after two years of regrowth (Gehring et al., 1999). Almost each of the 7 individually measured tree species responded to a different combination of nutrients in the latter study. This led to shifts in species contribution to overall biomass in some fertilizer treatments, depending on the responsiveness of the respective species, thereby revealing a potential mechanism by which nutrient availability may affect species composition during succession (Siddique et al., 2010).

A clearer pattern for nutrient limitation during secondary succession emerged from two adjacent studies in the Brazilian Amazon (Davidson & Martinelli, 2009). The earlier fertilization experiment reported a stronger stimulation of tree growth in early secondary forests in N fertilizer treatments (Davidson et al., 2004), and the following chronosequence study found various indications of a more conservative N cycle early in succession, including lower nitrate concentrations, lower nitrous oxide (N_2O) emissions and lower litterfall N:P ratios compared to late-successional and mature forests (Davidson et al., 2007). The authors suggested that this points to an intermittent N limitation early in succession that reverts to P limitation as forests grow older, a concept in line with the model predictions mentioned earlier (Nagy et al., 2017). Overall, there is experimental evidence that nutrient availability can limit biomass regrowth and

potentially affect the species composition of tropical secondary forests. The identity of limiting nutrients seems to differ for individual tree species and may further depend on the stage of regrowth, with indications for a shift from N to P limitation during succession.

3. Successional trends in nutrient cycling

3.1. The nitrogen cycle

Changes in the N cycle and the proposed shift from N to P limitation are driven by high N losses during deforestation and subsequent land-use, redistribution from soil N pools into plant biomass and inputs via atmospheric N fixation. Deforestation can lead to significant losses of N through a combination of vegetation burning or mechanic removal of biomass from the site, soil erosion, hydrological leaching and gaseous N losses (Davidson & Martinelli, 2009; Houghton, 2005; Kauffman et al., 2009). Especially biomass burning can lead to strong initial N losses, ranging between 51 and 62% of the original aboveground biomass N. Once vegetation cover is removed leaching of nutrients from the degraded sites can be increased as an effect of reduced evapotranspiration (Williams & Melack, 1997), and due to increased concentrations of nutrients in runoff of the cleared forest sites (Bringhurst & Jordan, 2015). Denitrification can lead to further soil N losses after land-use change. Different studies reported elevated N_2O emissions of young pastures compared to primary forests. As nitrate availability decreases with increasing pasture age emissions decrease to levels below primary forests, showing that denitrification can quickly use up the flush of inorganic N observed after forest clearing (Melillo et al., 2001; Neill et al., 2005). Decreased denitrification rates in early and mid-successional forests may then be the result of strong competition between microbes and plants for inorganic N at the time where aboveground biomass accrual is highest. A recent study showed that up to 40 years into regrowth nitrification rates remained more than 50% lower than in primary forests, even though N-mineralization rates were intermittently higher (Figueiredo et al., 2019). This suggests that high plant N demand can lead to a conservative N cycle as often observed early in succession, keeping nitrate

levels and thus potential N losses low.

Generally, plants can access N for regrowth from soil N pools via N mineralization or from the atmosphere via symbiotic N fixation (Davidson & Martinelli, 2009). Some studies using mass balance equations concluded that most of the N taken up by tropical trees in early succession may come from soil N pools (Davidson et al., 2018; Russell & Raich, 2012), whereas others highlighted the importance of symbiotic N fixation (Batterman et al., 2013; Gehring et al., 2005). In particular, Batterman et al. (2013) found that relative N fixer (legume) abundance, N fixation rates and root nodulation per hectare were highest early in succession, where N fixing species showed growth rates 9-times higher than non-fixers. They calculated that N fixation provided more than 50% of the N needed for biomass build-up during the first 12 years of succession. Overall, the relative contribution of mineralization and N fixation for forest recovery likely depends on local site conditions, species composition and climate (Powers & Marín-Spiotta, 2017). For example, the relative legume abundance is higher in tropical dry forest succession than in wet forest succession (Gei et al., 2018).

As secondary forests grow older they often show signs of a more leaky N cycle, suggesting an alleviation of an initial N limitation (Davidson et al., 2007). This concept is corroborated by a set of different soil and plant measurements from independent studies as summarized by Sullivan et al. (2019). Nitrification rates, soil nitrate concentrations, N_2O emissions and foliar and litter N:P ratios tend to increase with succession (Davidson et al., 2007; Figueiredo et al., 2019; Huang et al., 2012; Hayashi et al., 2012; Robertson & Tiedje, 1988; Sullivan et al., 2019), while the initial growth-advantage of N fixers and the proportion of nodulated legumes decrease (Batterman et al., 2013; Gehring et al., 2005). In conclusion, deforestation can lead to initial losses of N, and early secondary forests have shown signs of N limitation. During succession plants access N from soil pools and the atmosphere, and late successional forests tend to revert to a more open N cycle.

3.2. The phosphorus cycle

In contrast to N, P losses through deforestation are usually much lower, and soil pools represent the only significant source of P for regenerating plants. Although biomass

burning can remove up to 32% of aboveground biomass P from degraded sites, this only represents less than 10% of the combined above- and belowground P, since most of P is stored in soils (Kauffman et al., 1995). Furthermore, hydrological losses of the relatively less mobile P in cleared forests are lower than for N and usually appear in the form of soil total P when soil erosion happens during peak runoff events (Bringhurst & Jordan, 2015; Williams & Melack, 1997). However, even though P losses due to land-use change appear to be low, reductions in P pools may happen as a result of decreased atmospheric dust deposition. In the dry tropics of Mexico open field sites and 4-year-old secondary forests had almost 50% lower rates of atmospheric P deposition than 20-year-old secondary forests and primary forests, most likely as a result of decreased or missing canopy cover to trap dust particles (Lawrence et al., 2007). Still, there are no consistent trends in total soil P during tropical secondary succession, and both increases (Sullivan et al., 2019) and decreases in total soil P (Deng et al., 2017) have been reported in two recent meta-analyses, albeit the latter analysed data on tree plantations. In general, nutrient pumping from deeper soil layers may be an important mechanism to maintain P availability (Jobbágy & Jackson, 2001), as higher maximum fine root depths in secondary forests compared to primary forests have been found in sites that showed stable total P pools over multiple slash-and-burn cycles (Lawrence & Schlesinger, 2001).

In contrast to N, plants can access P for biomass build-up only from soil, where P exists in pools of different accessibility. Therefore, plant uptake during succession can lead to a redistribution of P between these pools. For example, four decades of forest regrowth on a low-P soil in South Carolina led to a depletion of mineral-associated organic and inorganic P pools while labile P remained unchanged (Richter et al., 2006). This shows that P pools which are considered as being of lower accessibility to plants can potentially maintain adequate P supplies throughout succession. Another pattern sometimes observed in the tropics is an increase in the relative contribution of organic P to total soil P with succession (Lawrence & Schlesinger, 2001; Zhang et al., 2016). However, there is an overall paucity of data on successional changes of P in the moist and wet tropics, which makes generalizations difficult. All in all, initial losses of P tend to be low. As plants take up P from soils during succession, deep rooting and pumping of P from deeper soil layers may replenish topsoil P pools. Plant uptake can further lead to a

restructuring of soil P pools, with the relative importance of organic P potentially increasing during tropical secondary succession.

4. Aim of the study

In this study we aimed to characterize changes in soil physicochemical, biogeochemical and microbial properties during tropical secondary succession, and to test how these changes affect soil P cycling. Previous studies have highlighted the prominent role of P as limiting nutrient in the tropics, and that changes in P pools can occur as forests recover. Yet, how microbes affect P cycling during succession, and the potential interactions of P with the C and N cycle have remained largely unknown. The study was conducted in a secondary forest chronosequence in a moist lowland tropical forest in Panama, including 5 active pastures, 5 old-growth forests and 53 secondary forest sites, ranging from 3 to approximately 40 years of age after abandonment of former cattle pastures. We measured soil physicochemical properties (pH, texture, Al/Fe oxides, cation exchange capacity, exchangeable Al^{3+} and Ca^{2+} etc.) as well as total, dissolved and microbial C and N, and related them to changes in total, available and microbial P, soil P sorption, gross P mobilization and hydrolytic soil enzyme activities.

5. References

Anderson, R. G., Canadell, J. G., Randerson, J. T., Jackson, R. B., Hungate, B. A., Baldocchi, D. D., … O'Halloran, T. L. (2011). Biophysical considerations in forestry for climate protection. *Frontiers in Ecology and the Environment*, *9*(3), 174–182. https://doi.org/10.1890/090179

Baccini, A., Goetz, S. J., Walker, W. S., Laporte, N. T., Sun, M., Sulla-Menashe, D., … Houghton, R. A. (2012). Estimated carbon dioxide emissions from tropical deforestation improved by carbon-density maps. *Nature Climate Change*, *2*(3), 182–185. https://doi.org/10.1038/nclimate1354

Bala, G., Caldeira, K., Wickett, M., Phillips, T. J., Lobell, D. B., Delire, C., & Mirin, A. (2007). Combined climate and carbon-cycle effects of large-scale deforestation. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(16), 6550–6555. https://doi.org/10.1073/pnas.0608998104

Barlow, J., Gardner, T. A., Araujo, I. S., Avila-Pires, T. C., Bonaldo, A. B., Costa, J. E., … Peres, C. A. (2007). Quantifying the biodiversity value of tropical primary, secondary, and plantation forests. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(47), 18555–18560. https://doi.org/10.1073/pnas.0703333104

Batterman, S. A., Hedin, L. O., Van Breugel, M., Ransijn, J., Craven, D. J., & Hall, J. S. (2013). Key role of symbiotic dinitrogen fixation in tropical forest secondary succession. *Nature*, *502*, 224–227. https://doi.org/10.1038/nature12525

Becknell, J. M., Kissing Kucek, L., & Powers, J. S. (2012). Aboveground biomass in mature and secondary seasonally dry tropical forests: A literature review and global synthesis. *Forest Ecology and Management*, *276*, 88–95. https://doi.org/10.1016/J.FORECO.2012.03.033

Bradshaw, C. J. A., Sodhi, N. S., Peh, K. S.-H., & Brook, B. W. (2007). Global evidence that deforestation amplifies flood risk and severity in the developing world. *Global Change Biology*, *13*(11), 2379–2395. https://doi.org/10.1111/j.1365-2486.2007.01446.x

Bringhurst, K., & Jordan, P. (2015). The impact on nutrient cycles from tropical forest to pasture conversion in Costa Rica. *Sustainable Water Resources Management*, *1*, 3–13. https://doi.org/10.1007/s40899-015-0003-x

Bruijnzeel, L. A. (2004). Hydrological functions of tropical forests: Not seeing the soil for the trees? *Agriculture, Ecosystems and Environment*, *104*(1), 185–228. https://doi.org/10.1016/j.agee.2004.01.015

Campo, J., Solis, E., & Gallardo, J. (2012). Effects of fertilisation on soil nutrient characteristics and the growth of tree stand in secondary seasonally dry tropical forests in Mexico. *Journal of Tropical Forest Science*, *24*(3), 419–426.

Chazdon, R. L. (2014). *Second growth : The promise of tropical forest regeneration in an age of deforestation*. Chicago; London: The University of Chicago Press.

Chazdon, R. L., Broadbent, E. N., Rozendaal, D. M. A., Bongers, F., & et al. (2016). Carbon sequestration potential of second-growth forest regeneration in the Latin American tropics. *Science Advances*, *2*, e1501639. https://doi.org/10.1126/sciadv.1501639

Chokkalingam, U., & de Jong, W. (2001). Secondary forest: a working definition and typology. *International Forestry Review*, *3*, 19–26.

Davidson, E.A., & Martinelli, L. A. (2009). Nutrient limitations to secondary forest regrowth. *Geophysical Monograph Series*, *186*, 299–309. https://doi.org/10.1029/2008GM000732

Davidson, Eric A., De Carvalho, C. J. R., Figueira, A. M., Ishida, F. Y., Ometto, J. P. H. B., Nardoto, G. B., … Martinelli, L. A. (2007). Recuperation of nitrogen cycling in Amazonian forests following agricultural abandonment. *Nature*, *447*, 995–998. https://doi.org/10.1038/nature05900

Davidson, Eric A., Markewitz, D., de O. Figueiredo, R., & de Camargo, P. B. (2018). Nitrogen fixation inputs in pasture and early successional forest in the Brazilian Amazon region: Evidence from a claybox mesocosm study. *Journal of Geophysical Research: Biogeosciences*, *123*(2), 712–721. https://doi.org/10.1002/2017JG004103

Davidson, Eric A, Carvalho, C. J. R. De, Vieira, I. C. G., Figueiredo, R. De, Moutinho, P., Ishida, F. Y., … Sabá, R. T. (2004). Nitrogen and phosphorus limitation of biomass growth in a tropical secondary forest. *Ecological Applications*, *14*(4), 150–163. https://doi.org/10.1890/01-6006

Deng, Q., McMahon, D. E., Xiang, Y., Yu, C.-L., Jackson, R. B., & Hui, D. (2017). A global meta-

analysis of soil phosphorus dynamics after afforestation. *New Phytologist*, *213*, 181–192. https://doi.org/10.1111/nph.14119

Dent, D. H., & Joseph Wright, S. (2009). The future of tropical species in secondary forests: A quantitative review. *Biological Conservation*, *142*(12), 2833–2843. https://doi.org/10.1016/j.biocon.2009.05.035

Dirzo, R., & Raven, P. H. (2003). Global state of biodiversity and loss. *Annual Review of Environment and Resources*, *28*, 137–167. https://doi.org/10.1146/annurev.energy.28.050302.105532

Eaton, J. M., & Lawrence, D. (2009). Loss of carbon sequestration potential after several decades of shifting cultivation in the Southern Yucatán. *Forest Ecology and Management*, *258*, 949–958. https://doi.org/10.1016/j.foreco.2008.10.019

Evaristo, J., & McDonnell, J. J. (2019). Global analysis of streamflow response to forest management. *Nature*, *570*(1), 455–461. https://doi.org/10.1038/s41586-019-1306-0

FAO. (2010). Global Forest Resources Assessment 2010. *FAO Forestry Paper*, *163*, 350 pp. https://doi.org/ISBN 978-92-5-106654-6

FAO. (2012). Global Forest Resources Assessment (FRA) 2015. Terms and definitions. *FRA Working Paper 180. FAO, Rome, Italy.*

Figueiredo, V., Enrich-Prast, A., & Rütting, T. (2019). Evolution of nitrogen cycling in regrowing Amazonian rainforest. *Scientific Reports*, *9*, 8538. https://doi.org/10.1038/s41598-019-43963-4

Gehring, C., Denich, M., Kanashiro, M., & Vlek, P. L. G. (1999). Response of secondary vegetation in Eastern Amazonia to relaxed nutrient availability constraints. *Biogeochemistry*, *45*, 223–241. https://doi.org/10.1007/BF00993001

Gehring, C., Vlek, P. L. G., De Souza, L. A. G., & Denich, M. (2005). Biological nitrogen fixation in secondary regrowth and mature rainforest of central Amazonia. *Agriculture, Ecosystems and Environment*, *111*, 237–252. https://doi.org/10.1016/j.agee.2005.06.009

Gei, M., Rozendaal, D. M. A., Poorter, L., Bongers, F., Sprent, J. I., Garner, M. D., … Powers, J. S. (2018). Legume abundance along successional and rainfall gradients in Neotropical forests. *Nature Ecology & Evolution*, *2*(7), 1104–1111. https://doi.org/10.1038/s41559-018-0559-6

Gibson, L., Lee, T. M., Koh, L. P., Brook, B. W., Gardner, T. A., Barlow, J., … Sodhi, N. S. (2011). Primary forests are irreplaceable for sustaining tropical biodiversity. *Nature*, *478*(7369), 378–381. https://doi.org/10.1038/nature10425

Gilroy, J. J., Woodcock, P., Edwards, F. A., Wheeler, C., Baptiste, B. L. G., Medina Uribe, C. A., … Edwards, D. P. (2014). Cheap carbon and biodiversity co-benefits from forest regeneration in a hotspot of endemism. *Nature Climate Change*, *4*, 503–507. https://doi.org/10.1038/nclimate2200

Hayashi, S. N., Guimarães, I. C. V., Reis, C. J. C., & Davidson, E. (2012). Linking nitrogen and phosphorus dynamics in litter production and decomposition during secondary forest succession in the eastern Amazon. *Boletim Do Museu Paraense Emílio Goeldi. Ciências Naturais*, *7*(3), 283–295.

Holdridge, L. R. (1947). Determination of world plant formations from simple climatic data. *Science*, *105*(2727), 367–368.

Hosonuma, N., Herold, M., De Sy, V., De Fries, R. S., Brockhaus, M., Verchot, L., … Romijn, E.

(2012). An assessment of deforestation and forest degradation drivers in developing countries. *Environmental Research Letters*, *7*, 044009. https://doi.org/10.1088/1748-9326/7/4/044009

Houghton, R. A. (2005). Tropical deforestation as a source of greenhouse gas emissions. In P. Moutinho & S. Schwartzman (Eds.), *Tropical Deforestation and Climate Change*. Belem: IPAM.

Huang, W. J., Zhou, G. Y., & Liu, J. X. (2012). Nitrogen and phosphorus status and their influence on aboveground production under increasing nitrogen deposition in three successional forests. *Acta Oecologica*, *44*, 20–27. https://doi.org/10.1016/j.actao.2011.06.005

Jakovac, C. C., Peña-Claros, M., Kuyper, T. W., & Bongers, F. (2015). Loss of secondary-forest resilience by land-use intensification in the Amazon. *Journal of Ecology*, *103*, 67–77. https://doi.org/10.1111/1365-2745.12298

Jobbágy, E. G., & Jackson, R. B. (2001). The distribution of soil nutrients with depth: Global patterns and the imprint of plants. *Biogeochemistry*, *53*(1), 51–77. https://doi.org/10.1023/A:1010760720215

Kauffman, J. B., Cummings, D. L., Ward, D. E., & Babbitt, R. (1995). Fire in the Brazilian Amazon: 1. Biomass, nutrient pools, and losses in slashed primary forests. *Oecologia*, *104*(4), 397–408. https://doi.org/10.1007/BF00341336

Kauffman, J. B., Hughes, R. F., & Heider, C. (2009). Carbon pool and biomass dynamics associated with deforestation, land use, and agricultural abandonment in the neotropics. *Ecological Applications*, *19*(5), 1211–1222. https://doi.org/10.1890/08-1696.1

Lawrence, D., D'Odorico, P., Diekmann, L., Delonge, M., Das, R., & Eaton, J. (2007). Ecological feedbacks following deforestation create the potential for a catastrophic ecosystem shift in tropical dry forest. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(52), 20696–20701. https://doi.org/10.1073/pnas.0705005104

Lawrence, D., & Schlesinger, W. H. (2001). Changes in soil phosphorus during 200 years of shifting cultivation in Indonesia. *Ecology*, *82*(10), 2769–2780.

Lawrence, D., & Vandecar, K. (2015). Effects of tropical deforestation on climate and agriculture. *Nature Climate Change*, *5*(1), 27–36. https://doi.org/10.1038/nclimate2430

Lennox, G. D., Gardner, T. A., Thomson, J. R., Ferreira, J., Berenguer, E., Lees, A. C., … Barlow, J. (2018). Second rate or a second chance? Assessing biomass and biodiversity recovery in regenerating Amazonian forests. *Global Change Biology*, *24*(12), 5680–5694. https://doi.org/10.1111/gcb.14443

Lewis, S. L., Edwards, D. P., & Galbraith, D. (2015). Increasing human dominance of tropical forests. *Science (New York, N.Y.)*, *349*(6250), 827–832. https://doi.org/10.1126/science.aaa9932

Locatelli, B., Catterall, C. P., Imbach, P., Kumar, C., Lasco, R., Marín-Spiotta, E., … Uriarte, M. (2015). Tropical reforestation and climate change: Beyond carbon. *Restoration Ecology*, *23*(4), 337–343. https://doi.org/10.1111/rec.12209

Lohbeck, M., Poorter, L., Martinez-Ramos, M., & Bongers, F. (2015). Biomass is the main driver of changes in ecosystem process rates during tropical forest succession. *Ecology*, *96*(5), 1242–1252. https://doi.org/10.1890/14-0472.1

Malhi, Y., Gardner, T. A., Goldsmith, G. R., Silman, M. R., & Zelazowski, P. (2014). Tropical forests

in the Anthropocene. *Annual Review of Environment and Resources*, *39*, 125–159. https://doi.org/10.1146/annurev-environ-030713-155141

Markewitz, D., Figueiredo, R. de O., de Carvalho, C. J. R., & Davidson, E. A. (2012). Soil and tree response to P fertilization in a secondary tropical forest supported by an oxisol. *Biology and Fertility of Soils*, *48*(6), 665–678. https://doi.org/10.1007/s00374-011-0659-9

Martin, P. A., Newton, A. C., & Bullock, J. M. (2013). Carbon pools recover more rapidly than plant biodiversity in secondary tropical forests. *Proceedings of the Royal Society B*, *280*, 20132236. https://doi.org/10.1098/rspb.2013.2236

Melillo, J. M., Steudler, P. A., Feigl, B. J., Neill, C., Garcia, D., Piccolo, M. C., … Tian, H. (2001). Nitrous oxide emissions from forests and pastures of various ages in the Brazilian Amazon. *Journal of Geophysical Research*, *106*(D24), 34179–34188. https://doi.org/10.1029/2000JD000036

Nagy, R. C., Rastetter, E. B., Neill, C., & Porder, S. (2017). Nutrient limitation in tropical secondary forests following different management practices. *Ecological Applications*, *27*(3), 734–755. https://doi.org/10.1002/eap.1478

Neeff, T., Lucas, R. M., Santos, J. R. dos, Brondizio, E. S., & Freitas, C. C. (2006). Area and age of secondary forests in Brazilian Amazonia 1978–2002: An empirical estimate. *Ecosystems*, *9*(4), 609–623. https://doi.org/10.1007/s10021-006-0001-9

Neill, C., Steudler, P. A., Garcia-Montiel, D. C., Melillo, J. M., Feigl, B. J., Piccolo, M. C., & Cerri, C. C. (2005). Rates and controls of nitrous oxide and nitric oxide emissions following conversion of forest to pasture in Rondônia. *Nutrient Cycling in Agroecosystems*, *71*(1), 1–15. https://doi.org/10.1007/s10705-004-0378-9

Norden, N., Angarita, H. A., Bongers, F., Martínez-Ramos, M., Cerda, I. G. la, Breugel, M. van, … Chazdon, R. L. (2015). Successional dynamics in Neotropical forests are as uncertain as they are predictable. *Proceedings of the National Academy of Sciences*, *112*(26), 8013–8018. https://doi.org/10.1073/PNAS.1500403112

Ogden, F. L., Crouch, T. D., Stallard, R. F., & Hall, J. S. (2013). Effect of land cover and use on dry season river runoff, runoff efficiency, and peak storm runoff in the seasonal tropics of Central Panama. *Water Resources Research*, *49*, 8443–8462. https://doi.org/10.1002/2013WR013956

Pan, Y., Birdsey, R., Fang, J., Houghton, R., Kauppi, P., Kurz, W., … Hayes, D. (2011). A large and persistent carbon sink in the world's forests. *Science*, *333*(6045), 988–993.

Poorter, L., Bongers, F., Aide, T. M., Almeyda Zambrano, A. M., Balvanera, P., Becknell, J. M., … Rozendaal, D. M. A. (2016). Biomass resilience of Neotropical secondary forests. *Nature*, *530*, 211–214. https://doi.org/10.1038/nature16512

Powers, J. S., & Marín-Spiotta, E. (2017). Ecosystem processes and biogeochemical cycles in secondary tropical forest succession. *Annual Review of Ecology, Evolution, and Systematics*, *48*, 497–519. https://doi.org/10.1146/annurev-ecolsys-110316-022944

Richter, D. D., Allen, H. L., Li, J., Markewitz, D., & Raikes, J. (2006). Bioavailability of slowly cycling soil phosphorus: Major restructuring of soil P fractions over four decades in an aggrading forest. *Oecologia*, *150*, 259–271. https://doi.org/10.1007/s00442-006-0510-4

Robertson, G. P., & Tiedje, J. M. (1988). Deforestation alters denitrification in a lowland tropical rain forest. *Nature*, *336*(22), 756–759. https://doi.org/10.1038/336756a0

Robledo, C., Fischler, M., & Patiño, A. (2004). Increasing the resilience of hillside communities in Bolivia. *Mountain Research and Development*, *24*, 14–18. https://doi.org/10.1659/0276-4741(2004)024[0014:ITROHC]2.0.CO;2

Rozendaal, D. M. A., Bongers, F., Aide, T. M., Alvarez-Dávila, E., Ascarrunz, N., Balvanera, P., … Poorter, L. (2019). Biodiversity recovery of Neotropical secondary forests. *Science Advances*, *5*(3), eaau3114. https://doi.org/10.1126/sciadv.aau3114

Russell, A. E., & Raich, J. W. (2012). Rapidly growing tropical trees mobilize remarkable amounts of nitrogen, in ways that differ surprisingly among species. *Proceedings of the National Academy of Sciences*, *109*(26), 10398–10402. https://doi.org/10.1073/PNAS.1204157109

Seymour, F., & Harris, N. L. (2019). Reducing tropical deforestation. *Science*, *365*(6455), 756–757.

Siddique, I., Vieira, I. C. G., Schmidt, S., Lamb, D., Carvalho, C. J. R., Figueiredo, R. de O., … Davidson, E. A. (2010). Nitrogen and phosphorus additions negatively affect tree species diversity in tropical forest regrowth trajectories. *Ecology*, *91*(7), 2121–2131. https://doi.org/10.1890/09-0636.1

Stegen, J. C., Swenson, N. G., Enquist, B. J., White, E. P., Phillips, O. L., Jørgensen, P. M., … Núñez Vargas, P. (2011). Variation in above-ground forest biomass across broad climatic gradients. *Global Ecology and Biogeography*, *20*(5), 744–754. https://doi.org/10.1111/j.1466-8238.2010.00645.x

Sullivan, B. W., Nifong, R. L., Nasto, M. K., Alvarez-Clare, S., Dencker, C. M., Soper, F. M., … Cleveland, C. C. (2019). Biogeochemical recuperation of lowland tropical forest during succession. *Ecology*, *100*(4), e02641. https://doi.org/10.1002/ecy.2641

Toledo, M., Poorter, L., Peña-Claros, M., Alarcón, A., Balcázar, J., Leaño, C., … Bongers, F. (2011). Climate is a stronger driver of tree and forest growth rates than soil and disturbance. *Journal of Ecology*, *99*, 254–264. https://doi.org/10.1111/j.1365-2745.2010.01741.x

Turner, B. L., Brenes-Arguedas, T., & Condit, R. (2018). Pervasive phosphorus limitation of tree species but not communities in tropical forests. *Nature*, *555*, 367–370. https://doi.org/10.1038/nature25789

van Breugel, M., Craven, D., Lai, H. R., Baillon, M., Turner, B. L., & Hall, J. S. (2019). Soil nutrients and dispersal limitation shape compositional variation in secondary tropical forests across multiple scales. *Journal of Ecology*, *107*, 566–581. https://doi.org/10.1111/1365-2745.13126

van Breugel, M., Hall, J. S., Craven, D., Bailon, M., Hernandez, A., Abbene, M., & Van Breugel, P. (2013). Succession of ephemeral secondary forests and their limited role for the conservation of floristic diversity in a human-modified tropical landscape. *PLoS ONE*, *8*(12). https://doi.org/10.1371/journal.pone.0082433

van der Werf, G. R., Morton, D. C., DeFries, R. S., Olivier, J. G. J., Kasibhatla, P. S., Jackson, R. B., … Randerson, J. T. (2009). CO2 emissions from forest loss. *Nature Geoscience*, *2*(11), 737–738. https://doi.org/10.1038/ngeo671

Vitousek, P. M., Porder, S., Houlton, B. Z., & Chadwick, O. A. (2010). Terrestrial phosphorus limitation: Mechanisms, implications, and nitrogen-phosphorus interactions. *Ecological Applications*, *20*(1), 5–15. https://doi.org/10.1890/08-0127.1

Walker, T. W., & Syers, J. K. (1976). The fate of phosphorus during pedogenesis. *Geoderma*, *15*, 1–19. https://doi.org/10.1016/0016-7061(76)90066-5

Williams, M. R., & Melack, J. M. (1997). Solute export from forested and partially deforested chatchments in the central Amazon. *Biogeochemistry*, *38*, 67–102. https://doi.org/10.1023/A:1005774431820

Wright, S. J., Turner, B. L., Yavitt, J. B., Harms, K. E., Kaspari, M., Tanner, E. V. J., … Garcia, M. N. (2018). Plant responses to fertilization experiments in lowland, species-rich, tropical forests. *Ecology*, *99*(5), 1129–1138. https://doi.org/10.1002/ecy.2193

Yang, X., & Post, W. M. (2011). Phosphorus transformations as a function of pedogenesis: A synthesis of soil phosphorus data using Hedley fractionation method. *Biogeosciences*, *8*, 2907–2916. https://doi.org/10.5194/bg-8-2907-2011

Zarin, D. J., Davidson, E. A., Brondizio, E., Vieira, I. C., Sá, T., Feldpausch, T., … Denich, M. (2005). Legacy of fire slows carbon accumulation in Amazonian forest regrowth. *Frontiers in Ecology and the Environment*, *3*(7), 365–369. https://doi.org/10.1890/1540-9295(2005)003[0365:LOFSCA]2.0.CO;2

Zhang, H., Shi, L., Wen, D., & Yu, K. (2016). Soil potential labile but not occluded phosphorus forms increase with forest succession. *Biology and Fertility of Soils*, *52*, 41–51. https://doi.org/10.1007/s00374-015-1053-9

Manuscript

1. Introduction

Tropical forests store more carbon (C) in plant biomass than any other biome and host the highest eukaryotic diversity on earth (Dirzo & Raven, 2003; Pan et al., 2011), but are increasingly threatened by human activity such as logging and conversion of forests into agricultural land (Hosonuma et al., 2012; Seymour & Harris, 2019). When previously degraded land is left abandoned or purposefully set aside for reforestation, secondary forests can regenerate, and today more than 50% of the tropical forested area is covered by secondary forests (FAO, 2010). The ability of secondary forests to restore ecosystem services as provided by primary forests depends on the level of plant biomass recovery (Gilroy et al., 2014). Forest regrowth usually proceeds fast with an average time of 66 years to recover 90% of original plant biomass, albeit high variation exists between sites regarding the speed of recovery and its overall magnitude (Poorter et al., 2016). A myriad of factors can affect secondary forest regrowth in the tropics (reviewed in Powers & Marín-Spiotta, 2017), including climate (Poorter et al., 2016), land-use history (Eaton & Lawrence, 2009; Jakovac et al., 2015; Zarin et al., 2005) and soil fertility (Nagy et al., 2017). Recently, the notion that nutrient availability constrains the speed of biomass regrowth in secondary succession has gained more attention, owing to the higher response of tropical secondary forests in fertilization experiments compared to primary forests (Wright et al., 2018).

Various fertilization studies found different limiting nutrients depending on plant species identity and successional stage (Campo et al., 2012; Gehring et al., 1999; Siddique et al., 2010). Over the recent years a pattern has emerged in the wet tropics in which nitrogen (N) limitation dominates early in succession but becomes alleviated as succession progresses (Davidson & Martinelli, 2009; Davidson et al., 2007; Davidson et al., 2004). Various lines of evidence have corroborated this hypothesis. Nitrification rates, soil nitrate concentrations, nitrous oxide emissions and foliar and litter N:phosphorus ratios

tend to increase with succession (Davidson et al., 2007; Figueiredo et al., 2019; Huang et al., 2012; Hayashi et al., 2012; Robertson & Tiedje, 1988; Sullivan et al., 2019), while the initial growth-advantage of N fixers and the proportion of nodulated legumes decrease (Batterman et al., 2013; Gehring, Vlek, De Souza, & Denich, 2005). The main cause for this intermittent N limitation is that relatively more N compared to phosphorus (P) is lost during deforestation and subsequent land-use (Bringhurst & Jordan, 2015; Davidson & Martinelli, 2009; Kauffman et al., 1995), but that these losses are compensated for during secondary forest succession due to high rates of symbiotic N fixation by legumes early in succession (Batterman et al., 2013). While Davidson et al. (2007) suggested that this points to a reversion to P limitation with progressing succession, experimental evidence for this is scarce from a P perspective.

In the strongly-weathered, acidic soils that cover the majority of lowland tropical forests much of the present P is occluded in soil minerals or locked up in organic matter and is hence considered poorly-available for plant uptake (Walker & Syers, 1976; Yang & Post, 2011). Under these conditions P can limit tropical tree growth and thereby constrain the C sink strength of tropical forests (Fleischer et al., 2019; Turner et al., 2018; Wang et al., 2015). Furthermore, current tropical tree species distribution and thus community assembly during succession can be affected by soil P status (Condit et al., 2013; van Breugel et al., 2019). At the same time, forest regrowth itself can change soil P pools by redistribution into plant biomass and by pumping P from deeper layers into topsoil via deep roots (Lawrence & Schlesinger, 2001; Richter et al., 2006), and could thereby feedback on the aforementioned processes.

Trajectories of soil P during succession seem to be highly context dependent, with no consistent trends for labile P (Powers & Marín-Spiotta, 2017), and two meta-analyses showing contrasting responses i.e. increases or decreases in soil total P, respectively (Deng et al., 2017; Sullivan et al., 2019). Richter et al. (2006) found a depletion of organic P and P pools associated with Fe- and Al-oxides after four decades of forest regrowth in South Carolina, showing that these slowly-cycling pools can become important sources of P during forest development. Microbes can assist plants by solubilizing P from sources that may be unavailable otherwise (Richardson & Simpson, 2011), but their role in P cycling during secondary succession is poorly understood. For example, microbes can

access and move P from mineral-associated pools to the biological cycle (He & Zhu, 1998). Particularly in the tropics, Fe- and Al-oxides represent major sorption sites for P in soils and thereby control abiotic P immobilization and constrain diffusive P fluxes (Goldberg & Sposito, 1984; Sollins et al., 1988), but the interaction between metal oxides and P in tropical secondary succession has not been studied yet. Tropical forest soils experience recurrent periods of anoxia during which microbial Fe-reduction can solubilize P from Fe-oxides and increase short-term P availability for microbes and plants (Chacon et al., 2006; Lin et al., 2018; Maranguit et al., 2017; Peretyazhko & Sposito, 2005). The magnitude of such reduction events depends strongly on microbial activity and inputs of plant labile C (Khan et al., 2019; Liptzin & Silver, 2009). Since both of these factors are expected to change with forest regrowth (Lohbeck et al., 2015; Raich et al., 2014), they could potentially feedback on the availability of mineral-associated P pools during succession via changes in Fe redox-activity.

While microbes can positively affect plant P supply, they are also strong competitors for P and can immobilize significant amounts of P in their biomass (L. P. Olander & Vitousek, 2005; Turner et al., 2013). Since microbial C and P are usually well correlated (Cleveland & Liptzin, 2007; Xu et al., 2013), any net increase in microbial biomass could lock up more P in microbes and thus interact with or promote plant P limitation during tropical secondary forest succession. Forest regrowth can affect microbial biomass primarily by changes in soil pH (Berthrong et al., 2009; Lauber et al., 2008), microclimate (Lebrija-Trejos et al., 2011; Serna-Chavez et al., 2013), or plant C inputs and soil organic C (Cleveland & Liptzin, 2007; Raich et al., 2014; Zheng et al., 2019). Increases in microbial biomass have been observed during tropical succession (Liu et al., 2012; Yokoyama et al., 2017; Zheng et al., 2019), yet soil microbial biomass P has been rarely measured itself. In addition to changes in nutrient pools, the activities of extracellular enzymes can serve as indicators for potential changes in microbial nutrient limitation that are hypothesized to occur during succession (Davidson et al., 2007). Microbes as well as plants produce extracellular phosphatases that cleave inorganic P from organic sources, rendering P available for uptake (Nannipieri et al., 2011). Since phosphatases are produced in response to P availability and demand (Cabugao et al., 2017; Yokoyama et al., 2017), their activities are often used to deduce nutrient limitations and were

demonstrated to be particularly high in activity in P-poor tropical forests (Waring et al., 2014).

In this study we aimed to get a more detailed, mechanistic understanding of how soil P cycling develops during tropical secondary forest succession by investigating interactions of soil microbes and metal oxides with P availability. Specifically, we wanted to address the following questions:

i) How do amorphous metal oxides interact with soil P immobilization, gross P mobilization and P pools at different stages of succession?

ii) Does microbial biomass increase and lead to higher biotic immobilization of P in microbial biomass during succession?

iii) Do indicators of N and P cycling (total and labile pools of N and P, extracellular enzyme activities, gross P mobilization) suggest aggravating P limitation with increasing successional trajectory?

In order to answer these questions, we took advantage of a highly replicated secondary forest succession chronosequence established in a moist tropical lowland forest region in Central Panama, spanning 63 sites including pastures, secondary forests differing in time of recovery from cattle pasture, and old-growth forests (OGF). Along the chronosequence we measured soil physicochemical properties (soil pH, texture, amorphous Al-/Fe-oxides, cation exchange capacity, exchangeable Al and Ca etc.) as well as total, dissolved and microbial biomass C and N, and related them to changes in total, available and microbial biomass P, soil P sorption, gross P mobilization and hydrolytic soil enzyme activities.

2. Materials and methods

Study area

The study is part of the Agua Salud Project supervised by the Smithsonian Tropical Research Institute, Panama. The study area has been extensively described in previous

studies (Batterman et al., 2013; Neumann-Cosel et al., 2011; Ogden et al., 2013; Van Breugel et al., 2013). The study area is located in the central Panama Canal watershed (9°13'N, 79°47'W, 330 m asl) and consists of a mosaic of small agricultural fields, cattle pastures, fallow vegetation and secondary forests of various ages, with cattle ranching as the predominant management form. The area receives approximately 2700 mm of rain per year, and the dry season lasts from December until early May (Ogden et al., 2013). The mean daily temperature on nearby Barro Colorado Island is 27 °C (Windsor, 1990). Soils mainly consist of strongly weathered, nutrient-poor Oxisols and Inceptisols developed from basaltic bedrock (van Breugel et al., 2019). The topography consists of steep slopes interspersed with streams that drain into the Panama Canal (Neumann-Cosel et al., 2011). Within the study area the Smithsonian Tropical Research Institute manages 664 ha of land of which approximately 530 ha were covered by fallow vegetation and secondary forests in 2008 (Van Breugel et al., 2013).

In 2013, 54 randomly selected sites were established along slopes of former cattle pastures or agricultural fields in order to form a transect of secondary forests differing in age of regrowth (Van Breugel et al., 2013). The age of the forests on the established sites was determined by interviews with former landowners and local residents and ranged from 10 to 39 years of regrowth in 2018, at the time this study was conducted. The transect includes five OGF for which exact ages could not be determined. All secondary forest sites originate from cattle pastures before abandonment. In rare cases pastures may have been intermittently converted to small-scale cultivation and then back to pastures. The distance between sites is 2,158 ± 1,293 m (mean ± SD), and each site consists of two 20x50 m plots oriented perpendicular to the slope, one on the upper section of the slope and one on the lower section of the slope. For the present study we selected only upslope plots of 53 sites of the established secondary forest network. One site had experienced severe loss of vegetation and was excluded from our study. We further included five 3-year-old forest plots (40x40 m) that are part of another experiment in the area as well as 5 active cattle pasture sites in the area to reach a total number of 63 sites. A chronosequence approach is used in this study, where differences between forest sites differing in time of regrowth are expected to represent differences arising during secondary succession. Plant communities in the successional forests are characterized by high heterogeneity and low local dominance of individual species. Only

6 out of 526 observed species made up more than 5% of all plants in more than 10% of all plots (Van Breugel et al., 2013). Some of the more frequent plant species include *Vernonanthura patens*, *Piper aduncum*, and *Conostegia speciose* in early succession, and *Swartzia simplex*, *Lacistema aggregatum*, *Pachira sessilis*, *Casearia arborea* and *Trichospermum galleottii* in late succession. Aboveground biomass data for the 53 sites of the secondary forest transect stem from a census in 2017 and were provided by Michiel van Breugel. Aboveground biomass of pastures and 3-year-old forests was estimated from Van Breugel et al. (2013).

Soil sampling

For every site 12 samples of the upper 10 cm of mineral soil were taken with a cylindrical soil corer (2.54 cm diameter), after removing the litter layer, and bulked together in one polyethylene bag to get one composite soil sample per site (n=63). In the 53 20x50 m secondary forest plots two soil samples were taken at 15 and 35 m down the center line and five samples each along the parallel lines 5 m away from the center line at 5, 15, 25, 35 and 45 m. The same sampling layout was used in the pasture plots where measuring tape was used as the center line along a randomly chosen upslope position within the pasture. Because the 40x40 m 3-year-old secondary succession plots consisted of dense shrublands soils were sampled in every corner and along the border lines 10 m away from every corner, in order to not disturb the regenerating vegetation. There was a buffer zone of at least 5 m to adjacent dirt roads, to minimize border effects. The soil samples were taken on 8 sampling days in August and September 2018. Per sampling day 7-9 sites were sampled and brought to the Soil Lab in the Earl S. Tupper Research Facility (Smithsonian Tropical Research Institute) in Panama City for immediate processing on the same day. Every sampling day included a random mix of site ages in order to minimize potential biases. Back in the lab the composite soil samples were homogenized by shaking and visible plant debris and stones were removed by hand. To prevent any storage effects, chloroform-fumigations and extractions of dissolved and microbial biomass C, N and P were done in the same day. The remaining fresh soil was kept at 4 °C for enzyme measurements within one week of sampling. Around 200 g of soil were air-dried at ambient temperature for two weeks and then passed through a 2

mm sieve after mechanical breakage of bigger soil aggregates. Subsamples of air-dried soil were ground in a ball mill.

Soil physicochemical properties

Soil water content was determined gravimetrically as the weight difference between fresh soils and oven-dried soils (105 °C, 48 h). Stable isotope composition and total content of C and N of air-dried, ground soil samples were measured after combustion using a Flash-HT elemental analyser coupled to a Thermo Scientific Delta V Advantage isotope ratio mass spectrometer (EA-IRMS) as described in Mackenzie et al. (2019). Soil pH was measured with a glass electrode on air-dried soil in deionized water and 0.01 M $CaCl_2$ (1:2 (w:v) soil dry weight:solution) and 0.1 M $BaCl_2$ (1:3 (w:v) soil dry weight:solution). Soil texture was determined on 3 g air-dried soil using the micro-pipette method without prior removal of organic matter (W. P. Miller & Miller, 1987). Exchangeable cations were measured in the 0.1 M $BaCl_2$ extracts by inductively-coupled plasma optical-emission spectrometry (ICP–OES) using an Optima 2100 (Perkin-Elmer Inc., Shelton, CT) after 10-fold dilution with 2% HNO_3 (Hendershot & Duquette, 1986). Cation exchange capacity (CEC) was calculated as the sum of exchangeable Al, Ca, Fe, K, Mg, Mn and Na. Percentage of base saturation was calculated by dividing the sum of exchangeable Ca, K, Mg and Na by CEC. Oxalate extractable Al (oxalate Al), Fe (oxalate Fe) and P (oxalate P) were measured on air-dried, ground soil. Soils were extracted in the dark in a 1:40 soil to solution ratio with a solution containing oxalic acid and ammonium oxalate adjusted to pH 3 (Courchesne & Turmel, 2008). Oxalate extractable elements were measured by ICP–OES after 5-fold dilution with 2% HNO_3. Oxalate Fe and oxalate Al mainly represent amorphous Fe- and Al-oxides (Parfitt & Childs, 1988).

Total P, resin P and microbial biomass P

Total P was determined in air-dried soil by ignition at 450 °C for 5 h and extraction with 0.5 M H_2SO_4 at a 1:40 soil to solution ratio for 18 h. Total inorganic P was determined as above, but in non-ignited soil aliquots, and total organic P calculated by difference.

Phosphorus in the extracts was measured colorimetrically at 610 nm using an Infinte M Nano Plus (Tecan Trading AG, Switzerland) after malachite green reaction with phosphomolybdate under acidic conditions (Angelo et al. , 2001). Microbial biomass P was measured by hexanol-fumigation and extraction with anion-exchange membranes following the protocol described in Turner & Romero (2010) and developed by Kouno et al. (1995). Resin strips (manufactured by BDH, Poole, UK; distributed by VWR International, West Chester, PA) were prepared by initially shaking in 0.5 M HCO_3 twice for 1 h. From each soil duplicate aliquots of 8 g fresh soil were prepared. One aliquot received 80 mL deionized water (unfumigated) and the other aliquot 80 mL deionized water and 1 mL hexanol (fumigated). Fumigated and unfumigated samples both received five resin strips (1x4 cm) and after shaking on a horizontal shaker for 20 h, resin strips were cleaned of soil with deionized water. Phosphate was eluted from the resin strips by shaking in 50 mL 0.25 M H_2SO_4 for 1 h and then quantified at 880 nm by automated molybdate colorimetry using a Lachat Quickchem 8500 (Hach Ltd, Loveland, CO, USA). Microbial biomass P was calculated as the difference between the P concentrations of fumigated and unfumigated samples, without using a conversion factor. Unfumigated samples represent resin P.

P immobilization and gross P fluxes

Net P immobilization and gross P fluxes were determined using a ^{33}P isotope pool dilution assay as described in Wanek et al. (2019). In this assay ^{33}P-phosphate is added to soils and the concentration and radioactivity of phosphate followed over time. The assay was performed on air-dried soil that was rewetted to 44% soil water content (average field moisture) by adding 20 mL deionized water to 50 g air-dried soil two weeks prior to the experiment. For abiotic process controls (sterile soils), batches of each soil were autoclaved (121 °C for 1 h) 96 h and 24 h prior to the start of the assay. Aliquots of 3 g rewetted soil were weighed into 50 mL polyethylene tubes, each three replicates for live soil and for sterile soil, each for time points 1 (t_1, 4 h) and 2 (t_2, 24 h), summing up to 12 aliquots per soil. The assay was started by adding 200 µL inorganic ^{33}P tracer solution (dilution of orthophosphoric acid P-33 radionuclide, 370 MBq/mL, American Radiolabelled Chemicals Inc., St. Louis, MO) to each soil sample, representing

an amendment of approximately 17 kBq per sample. Additionally, three recovery standards were prepared for each time point that received 200 µL ^{33}P tracer solution but did not contain soil. Samples were incubated at ambient temperature (~20 °C) for 4 h (t_1) and 24 h (t_2), respectively. The incubations were terminated by adding 15 mL 0.5 M $NaHCO_3$ and shaking on a horizontal shaker for 30 min. Samples were centrifuged at 5000 *g* for 2 min and passed through ash-free cellulose filters. Acidification of extracts was done by adding 0.3 mL 2.75 M H_2SO_4 to 3 ml filtrate. After CO_2 evolution stopped, 4 ml scintillation cocktail (Ultima Gold, Perkin Elmer) was added to each 250 µL acidified extract. Samples were thoroughly mixed and kept in the dark overnight prior to quantification of ^{33}P activity by liquid scintillation counting on a Tri-Carb 4910 TR (Perkin Elmer). The rest of the acidified extracts was kept at 4 °C prior to colorimetric determination of inorganic P (=^{31}P measurement) at 610 nm on a Multiskan GO microtiter plate reader (Thermo Scientific) after malachite green reaction with phosphomolybdate under acidic conditions (Angelo et al. , 2001). Changes in the ratio of ^{33}P to ^{31}P (=specific activity; in Bq μg^{-1} Pi) from t_1 to t_2 with respect to the changes in the overall inorganic P concentration were used to calculate gross P fluxes according to the isotope pool dilution equations below (Wanek et al. 2019).

$$\text{Gross influx: } GI = \frac{C_{t2} - C_{t1}}{t_2 - t_1} \times \frac{ln\left(\frac{SA_{t1}}{SA_{t2}}\right)}{ln\left(\frac{C_{t2}}{C_{t1}}\right)} \qquad \text{(Eq. 1)}$$

$$\text{Gross efflux: } GE = \frac{C_{t1} - C_{t2}}{t_2 - t_1} \times \left(1 + \frac{ln\left(\frac{SA_{t2}}{SA_{t1}}\right)}{ln\left(\frac{C_{t2}}{C_{t1}}\right)}\right) \text{ (Eq. 2)}$$

where t_1 and t_2 represent incubation time (4 and 24 h; in days), *C* the soil P_i concentration (in µg P_i g^{-1} soil dry weight), *SA* the specific activity (in Bq μg^{-1} P_i) and LN the natural logarithm. Gross influx in live soils represents gross Pi mobilization by biotic and abiotic processes (organic P mineralization and Pi desorption), gross efflux the rates of biotic and abiotic P immobilization (microbial Pi uptake and Pi sorption to the soil matrix). Gross P fluxes are expressed as µg P g^{-1} soil dry weight d^{-1}.

Net abiotic P immobilization was calculated on sterile soils as 100 minus the percentage of sample ^{33}P relative to recovery standard ^{33}P after 4 h (= t_1).

Dissolved and microbial biomass C and N

Microbial biomass C and N were measured by chloroform-fumigation extraction following standard procedures (Brookes et al., 1985; Vance et al., 1987). Duplicate samples of 25 g of each fresh soil were prepared. One aliquot was immediately extracted with 75 mL 0.5 M K_2SO_4 on a horizontal shaker for 1 h. Then the samples were centrifuged (15 min, 10.000 *g*), 20 mL of the supernatant transferred to new vials and stored at -20 °C for later analysis. The other aliquot was fumigated in a desiccator with chloroform-saturated atmosphere for 24 h before extraction with 0.5 M K_2SO_4. Dissolved organic C (DOC) and total dissolved N (TDN) in the extracts were analysed on a TOC-V_{CHN} analyzer (Shimadzu, Columbia, MD). Microbial biomass C and N were calculated by subtracting DOC (or TDN) of unfumigated samples from fumigated samples and dividing by a conversion factor of 0.45. The unfumigated samples represent dissolved organic C and total dissolved N concentrations in soils (Jones & Willett, 2006). Soil nitrate (NO_3^-) and ammonium (NH_4^+) were measured in the 0.5 M K_2SO_4 extracts of unfumigated soils on a Lachat Quikchem 8500 (Hach Ltd, Loveland, CO, USA).

Extracellular enzyme activities

Potential soil enzyme activities were measured with fluorigenic substrates (Marx et al., 2001), following the protocol described in Turner & Romero (2010). The following enzymes were measured with the respective substrates: (i) acid phosphomonoesterase (APM) with 4-methylumbelliferyl phosphate; (ii) phosphodiesterase (PD) with bis-(4-methylumbelliferyl) phosphate; (iii) ß-glucosidase (BG) with 4-methylumbelliferyl ß-D-glucopyranoside; (iv) N-acetyl-ß-D-glucosaminidase (NAG) with 4-methylumbelliferyl N-acetyl-ß-D-glucosaminide; and (v) L-Leucine aminopeptidase (LAP) with L-Leucine-7-amido-4-methylcoumarin. Substrates i-iv were dissolved in 0.4% methylcellosolve, (v) was dissolved in water. All substrates were prepared at 200 µM (100 µM final assay concentration) and kept at -20 °C until use. Soil slurries were prepared in 1 mM NaN_3 in water in a soil to solution ratio of 1:100 by stirring on a magnetic stirrer for 15 min. Assays were performed in black 96-well microtiter plates. A separate sample plate was prepared for every soil sample. In sample plates 100 µL 1 mM NaN_3 (quenched blank

wells), 100 µL 10 µM methylumbelliferone (MU) (quenched standard wells) and 100 µL substrates for APM, PD, BG and NAG (enzyme assay wells) were each pipetted in 8 replicates into wells containing 50 µL 200 mM acetate buffer (pH 5). For LAP MUF standards were substituted by 7-Amino-4-methylcoumarin (AMC) standards and 200 mM Trizma base buffer (pH 8) was added instead of acetate buffer. To start the assays, 50 µL soil slurry was added to each well. One control plate with the same layout as the sample plates but receiving 50 µL 1 mM NaN_3 instead of soil slurry was prepared. Plates were incubated at 26 °C (average study soil temperature). After 1 h the assays were terminated for APM, PD, BG and NAG by adding 50 µL modified universal buffer (MUB, pH 12; consisting of each 100 mM acetic acid, citric acid, maleic acid, boric acid and Trizma base in 500 mM sodium hydroxide). Wells for LAP determination were measured after 2 h of incubation without prior pH adjustment by MUB addition. Fluorescence intensity was determined at 360 nm excitation and 460 nm emission wavelength on a FLUOstar Optima multidetection plate reader (BMG Labtech, Offenburg, Germany) immediately after termination. After correcting fluorescence intensities of samples and standards for blank fluorescence, quenched and unquenched (control) standards were used to transform fluorescence units of samples into nmol MU or AMC released, respectively. According to Turner (2010), fluorescence is linear to at least 2 nmol MU (1 nmol MU used as standard here) at the assay conditions used in this study. All enzyme activities are expressed as nmol MU or AMC released g^{-1} soil dry weight min^{-1}.

Data analysis

In order to test for changes in soil parameters with secondary forest succession, regression analyses using forest age as predictor variable were performed. First non-linearity of responses was tested by polynomial curve fitting. If the quadratic term of the polynomial function was not significant, linear regressions were performed. Normality of residuals and variance homogeneity were checked graphically and, if necessary, data were square root-transformed, log-transformed and corrected for outliers, in this order. Since there was no information available on the exact age of the five OGF sites, these were excluded from the regression analysis and were instead compared to the five oldest secondary forests using the two-sample Student's t-test or the Mann-Whitney U-

test, if assumptions of parametric analysis were not met. Normality of data was checked graphically and using the Shapiro-Wilk test, after standardizing the old secondary forest and OGF data and pooling them. Homogeneity of variances was checked graphically and using the Levene test. Additionally, relationships between individual soil parameters were tested with linear or polynomial regressions as above. The five OGF sites were included in this analysis. All statistical analyses were performed in R-Studio version 3.4.4 (R Core Team), and graphs were created in SigmaPlot version 14 (Systat Software, Inc.).

3. Results

Changes during secondary succession

When testing for changes over the first 39 years of secondary succession, we found successional trends for many physicochemical and microbial properties. Significant trends were mostly linear, suggesting that they saturated between late-successional forests and OGF. Aboveground biomass increased strongly together with soil organic C (+26%; $p<0.001$), total N (+23%; $p<0.001$) and soil water content (+23%; $p<0.001$) (Fig. 1a-d). Soil $\delta^{13}C$ showed a saturating function and decreased with succession (-3.3‰; $p<0.001$) while soil $\delta^{15}N$ decreased linearly (-1.2‰; $p=0.007$) (Fig. 1e,f). While there were no clear trends for cation exchange capacity ($p=0.656$) and exchangeable Ca ($p=0.193$), soil pH decreased significantly (-0.4 units; $p=0.003$), accompanied by a 4-fold increase in exchangeable Al ($p=0.002$) and decreases in exchangeable K (-48%; $p=0.005$) and base saturation (-24%; $p=0.008$) (Fig. 2a-f). Soil texture ($p>0.25$ for sand, silt and clay content) and oxalate Al ($p=0.926$) didn't change over the first 38 years of forest regrowth (Fig. 3a-d). Oxalate Fe decreased significantly (-28%; $p=0.003$) together with decreases in oxalate P (-16%; $p=0.057$), total P (-17%; $p=0.067$), resin P (-41%; $p=0.011$) and gross P mobilization (-52%; $p=0.008$), while net abiotic P immobilization remained unaltered ($p=0.836$) (Fig. 3e,f and Fig. 4a,b,d,e). At the same time, microbial biomass P increased 2-fold ($p<0.001$) and the contribution of microbial biomass P to total P increased from 6% to 12% ($p<0.001$) (Fig. 4c,f).

Microbial biomass C and N showed a polynomial relationship with forest age. They both

increased early in succession but then microbial biomass N ($p<0.001$) saturated, and microbial biomass C ($p=0.004$) even decreased later in succession (Fig. 5a,d), leading to decreases in microbial C:N (-17%; $p=0.006$), C:P (-49%; $p<0.001$) and N:P (-41%; $p=0.004$) ratios (Fig.5b,c,e). In contrast to P, labile forms of C and N didn't show a clear pattern with succession and we found no effect of forest regrowth on dissolved organic C ($p=0.267$), total dissolved N ($p=0.327$), dissolved organic N ($p=0.910$) and NH_4^+ ($p=0.489$) (Fig. 6a-c,f). However, soil NO_3^- ($p<0.001$) and consequently dissolved inorganic N ($p=0.049$) showed a strong polynomial relationship with forest age. They both decreased from pasture to young secondary forests but increased to late-successional stages (Fig. 6d,e). When analysing extracellular enzyme activities, we found a strong relationship between forest age and phosphatase enzymes, with phosphomonoesterase activity increasing 2.3-fold ($p<0.001$) and phosphodiesterase activity increasing 2.1-fold ($p<0.001$) during succession (Fig. 7a,d). On the other hand, N-acetyl-glucosaminidase activity ($p=0.160$) and Leucine aminopeptidase activity ($p=0.868$) didn't change and ß-glucosidase activity even decreased slightly (-28%; $p=0.041$) (Fig. 7b,c,e).
This shifted the overall relative enzyme activities towards enzymes involved in P cycling, with 4-fold decreases in enzyme C:P ($p<0.001$) and 3.5-fold decreases in enzyme N:P ratios ($p<0.001$), but no change in enzyme C:N ratios ($p=0.219$) (Fig. 8d-f). Similar to extracellular enzyme activities, successional trends in total and extractable C, N and P ratios were strongest with regard to P. Extractable C:N ($p=0.447$) didn't change (Fig. 8a) and soil total C:N ($p<0.001$) (Fig. 9a) was only intermittently increased in mid-succession, while extractable C:P (2.6-fold increase; $p=0.004$) and N:P (3-fold increase; $p=0.002$) ratios (Fig. 8b,c) as well as soil total C:P (1.5-fold increase; $p<0.001$) and N:P (1.4-fold increase; $p<0.001$) ratios (Fig. 9b,c) increased steadily, showing that P decreased over the first 39 years of succession relative to C and N. Except for oxalate Al and clay content (Fig 3c,d), which were significantly and marginally lower in OGF, respectively, late-successional forests did not differ significantly from OGF, indicating that after 39 years of regrowth secondary forests had largely restored conditions of OGF.

Relationships between individual parameters

In order to explore how individual variables influenced each other during succession we performed linear regressions with selected soil parameters. Soil organic C was strongly positively related to soil water content (R^2=0.44; p<0.001) (Fig. 10a). Soil pH was strongly but non-linearly correlated to exchangeable cations, exchangeable Ca^{2+} (R^2=0.81; p<0.001) and base saturation (R^2=0.94; p<0.001) increased and exchangeable Al^{3+} (R^2=0.93; p<0.001) decreased with an increase in soil pH (Fig. 10b-d). Changes in P pools were better explained by oxalate-extractable Fe than oxalate-extractable Al. Oxalate-extractable Fe was positively correlated to total P (R^2=0.32; p<0.001) as well as resin P (R^2=0.20; p<0.001) (Fig. 11a,b), while oxalate-extractable Al showed a weaker correlation to total P (R^2=0.10; p=0.011) and no relation to resin P (p=0.875) (Fig. 11d,e). Furthermore, no relationship existed between oxalate-extractable Fe and Al (p=0.504) (Fig. 11f), but oxalate-extractable Fe was positively correlated to soil pH (R^2=0.26; p<0.001) (Fig. 11c). Microbial stoichiometry was characterized by stronger relationships between microbial biomass C and N (R^2=0.56; p<0.001) than microbial biomass C and P (R^2=0.20; p<0.001), as well as microbial biomass N and P (R^2=0.29; p<0.001) (Fig. 12a,b,d). Microbial biomass C itself was only weakly correlated to soil organic C (R^2=0.19; p=0.002) (Fig. 12e). Phosphomonoesterase activity was strongly affected by soil P-status and was negatively correlated to resin P (R^2=0.26; p<0.001) and positively correlated to soil C:P (R^2=0.35; p<0.001) (Fig. 12c,f). Correlations of soil pH, oxalate Fe, total dissolved N, resin P, microbial C, microbial P and phosphomonoesterase activity with a wider range of parameters can be found in Supplementary Table S1.

4. Discussion

Across the secondary forest succession in Agua Salud, Panama, soil organic C increased with age, reflecting the rapid accrual of forest aboveground biomass. At the same time soil acidity increased, paralleled by decreasing amorphous Fe-oxides and soil P fractions and the redistribution of soil P to microbial biomass. This clearly indicates strong

changes in nutritional constraints to plants and soil microbes along the successional trajectory.

Tropical secondary forests typically show high rates of aboveground biomass recovery (Poorter et al., 2016). Litterfall recovers to primary forest rates within only 10-20 years of regrowth (Brown & Lugo, 1990), and belowground C allocation and root biomass can even intermittently increase over pasture and primary forest levels (Raich et al., 2014; Zangaro et al., 2008). Therefore, plant-induced successional changes in soil conditions may result from increased C inputs in secondary forests compared to pastures (Powers & Marín-Spiotta, 2017). The increase in soil organic C has also been reported by others in the same region (Neumann-Cosel et al., 2011), though directions of soil organic C change can vary due to differences in climate, soil mineralogy or land-use history (Powers & Marín-Spiotta, 2017). Forest regrowth further led to a decrease of $\delta^{13}C$ over time, reflecting the shift of C inputs from relatively ^{13}C enriched organic matter from C_4 grasses in pastures to relatively ^{13}C depleted organic matter from C_3 trees in secondary forests (Wolf et al., 2011) and demonstrates rapid soil organic C turnover in tropical soils.

Changes in soil pH and exchangeable cations

We observed progressive soil acidification during secondary forest succession, with important implications on cation-exchange capacity, base saturation, P availability, Al toxicity, and microbial activity. Soil acidification has also been observed in some tropical successional studies (Bautista-Cruz & del Castillo, 2005) but not in others where soil pH only dropped intermittently (Smith et al., 2015; Wanek et al., unpublished data). Plant regrowth could have driven the observed pH decrease by a combination of factors, including high uptake rates of cations versus anions coupled with organic acid release to increase P solubilization in these low-P soils. Plant regrowth can decrease soil pH by the release of H^+ into the rhizosphere in order to counterbalance excess uptake of cations over anions and maintain electrochemical balance (Tang & Rengel, 2003). Leaves and wood of regenerating tropical trees represent a major sink for cations, especially Ca and K, but less so for anions (Fujii, 2014; Heineman et al., 2016). Therefore, soil acidification during afforestation has often been linked to the depletion of base cations in topsoils

(Berthrong et al., 2009). However, though soil pH decreased, base saturation was generally high and decreased non-significantly, with no change in exchangeable Ca, and only a slight decrease in exchangeable K. The decrease in soil pH may be driven by an imbalance in the uptake of soil NH_4^+ versus NO_3^-. Soil NO_3^- was almost undetectable in early successional forests and only increased late in succession, whereas NH_4^+ remained constant. The preferential uptake of NH_4^+ for plant N nutrition may have increased the ratio of cation over anion uptake and thus led to an increased efflux of H^+ into the rhizosphere. Furthermore, plants and microbes can release organic acids such as citric acid, maleic acid or oxalic acid into soils as a mechanism to increase the dissolution and desorption of P from soil minerals (Fujii, 2014; Johnson & Loeppert, 2006). Organic acids dissociate into their organic anion and H^+, depending on the soil solution pH, promoting soil acidification (Adeleke et al., 2017; Arcand & Schneider, 2006). In particular, under low P conditions, such as present in this study, increased rhizosphere acidification has been observed and linked to an increased release of organic acids in order to increase P solubilization (Ma et al., 2019).

Soil pH is also an important determinant of soil physicochemical properties and for instance affects the protonation and dissolution of metal oxides and thereby the solubility of ions and the ion-exchange capacity of soil minerals, particularly in variable-charge soils, such as those typically found in strongly-weathered tropical ecosystems (Deltombe & Pourbaix, 1958; Sollins et al., 1988; Stefánsson, 2007; Weil & Brady, 2017). In this study we found an increase in exchangeable Al to levels similar as exchangeable Ca with succession that closely followed the changes in soil acidification. Since H^+ can replace Al^{3+} from abiotic binding sites, the solubility of exchangeable Al^{3+} is highly dependent on pH and increases sharply below a soil pH of 5 (Kochian et al., 2004). Free Al^{3+} in soil solution is highly toxic for soil biota and can inhibit root growth and microbial proliferation (Illmer & Mutschlechner, 2004; Kochian et al., 2004). Thereby, the observed decrease in pH may lead to a state of Al-toxicity as is often observed in acidic soils (Fujii, 2014). In order to cope with Al-toxicity plants can detoxify Al^{3+} via complexation with organic ligands either internally or through the release of organic acids into the rhizosphere (Kochian et al., 2004). Since organic acid release can cause soil acidification but at the same time is involved in detoxification of Al and solubilization of P, plants may ameliorate the toxic conditions they create themselves. On the other

hand, this may inhibit growth of Al-sensitive microbes or restrict the activity of microbes that lack a detoxification mechanism to a narrower range around plant roots, where they benefit from detoxifying conditions.

Dynamics of amorphous Al- and Fe-oxides and P pools

Oxides and hydroxides of Fe and Al can strongly bind P via ligand-exchange reactions and are prevalent in strongly-weathered, acidic tropical soils (Goldberg & Sposito, 1984). The soils of the study sites were acidic, with high contents of Fe- and Al-oxides and low levels of total P, resin P and microbial P when compared to other areas in Central Panama (Turner et al., 2018; Turner & Engelbrecht, 2011) or global means (Xu et al., 2013; Yang & Post, 2011). Since much of the P in tropical soils can be associated with Fe- and Al-oxides (Esberg et al., 2010; Yang & Post, 2011), they significantly contribute to the low levels of available P and the often-suggested P limitation of lowland tropical forests (Vitousek et al., 2010). Oxalate Fe and oxalate Al mainly represent amorphous Fe- and Al-(hydr)oxides which possess a high surface area and thus a particularly high P sorption potential (Parfitt, 1989; Parfitt & Childs, 1988). Therefore, any change in oxalate Fe and Al during succession may have profound effects on P sorption strength and P pools. We found that oxalate Fe decreased during succession whereas oxalate Al remained constant and only decreased in OGF. Various pools of P (total P, oxalate P and resin P) were related to the changes in oxalate Fe (Fig. 11a,b and Supp. Table S1), but much weaker or not significantly with oxalate Al (Fig. 11d,e), and decreased in a similar manner, albeit only significantly in the case of resin P. The decrease in oxalate Fe and P pools was accompanied by a redistribution of P into microbial biomass and a decrease in gross P mobilization, while abiotic P immobilization did not change.

To our knowledge only one other study measured oxalate Fe and Al during secondary succession and found increases of both metal oxides late in succession (Richter et al., 2006). However, the study was conducted on low-P soils in South Carolina, and the behaviour of oxalate Fe and Al may differ markedly in wet tropical soils. Crystalline forms, such as goethite and hematite, are extracted in dithionite and have a lower surface area and affinity for P (Parfitt, 1989), but are relatively stable in soils (Schwertmann, 1991). On the other hand, amorphous Fe-oxides, such as ferrihydrite, are

extracted in oxalate and provide a large surface area for interactions with P, but are considered as more dynamic, metastable forms that can readily form from Fe in soil solution but also dissolve due to protonation, complexation with organic substances, and Fe reduction (Cornell et al., 1989; Cudennec & Lecerf, 2006; Schwertmann, 1991). Protonation can lead to the dissolution of metal oxides by adsorption of protons onto mineral surfaces which leads to polarization and weakening of Fe-O bonds. While the decrease in oxalate Fe was indeed related to soil acidification in this study (Fig. 11c), protonation has been described as the slowest process of Fe-oxide dissolution and may primarily act on geological timescales (Schwertmann, 1991).

On the other hand, biota including plants can release organic acids in order to mobilize Fe (and P) from oxides (see above) via complexation (Zanin et al., 2019). Since secondary forests rapidly build up large stocks of biomass, plant Fe uptake may partially account for the decrease in oxalate Fe. In a rough estimate using aboveground biomass data reported in this study and published data on plant wood and leaf Fe contents (Ancuceanu et al., 2015), we calculated that the uptake of Fe in plant biomass likely accounted for less than 1% of the decrease in oxalate Fe from pastures to OGF, highlighting that the plant Fe sink did not contribute strongly to the loss of oxalate Fe as observed in this study.

Thus, it is likely that increased Fe reduction, which is considered the fastest process involved in Fe-oxide dissolution (Schwertmann, 1991), was the main cause for oxalate Fe loss from topsoils during succession. Due to a combination of high biological activity and high rainfall, tropical forest soils can experience frequent changes in oxygen levels (Liptzin et al., 2011). Reduction of Fe^{3+} under anoxic conditions can dissolve and liberate Fe^{2+} from Fe-oxides, a process that can happen abiotically or can be driven by microbes. In addition to Fe-reducing bacteria, a whole range of other microbes, such as fermenters or methanogens, can reduce organic compounds under anoxic conditions which then pass on the electrons to Fe-oxides, thereby leading to their indirect reduction (Melton et al., 2014). The importance of this process for Fe cycling, particularly in ecosystems that experience periodic anoxia, has been demonstrated in many different studies (Chacon et al., 2006; Khan et al., 2019; Lin et al., 2018; Liptzin & Silver, 2009; Maranguit et al., 2017; Peretyazhko & Sposito, 2005). The Fe reduction potential depends strongly on labile C supply (Khan et al., 2019; Liptzin & Silver, 2009), and is thus believed to be higher in the

rhizosphere than in bulk soil (Fimmen et al., 2008; Weiss et al., 2004). The observed increase in plant biomass and soil organic C and in soil water content may have improved conditions for microbial activity and thus increased the Fe reduction potential in the studied secondary forest sites, leading to a gradual dissolution of oxalate Fe. In addition, the higher soil water content observed in older secondary forest soils could have increased the frequency or duration of anoxic periods. Owing to the strong association between P and Fe-oxides, the dynamics of Fe and P during redox cycles are intimately linked, and thus they typically show concerted increases and decreases during anoxic and oxic conditions (Lin et al., 2018). Since P can bind to other sorption sites once liberated, and free Fe^{2+} quickly re-oxidizes and reprecipitates under oxic conditions, their redox-induced changes are usually only short-lived and labile P therefore doesn't build up over time (Lin et al., 2018). However, during these short solubilization pulses P is available for biological uptake, and particularly microbes have been shown to be strong competitors for P with abiotic sorption sites under P-limiting conditions (Olander & Vitousek, 2005; Olander & Vitousek, 2004). Liptzin & Silver (2009) experimentally studied redox dynamics of Fe and P with C additions and found that after ~1 month repetitive anoxic/oxic cycles caused the transformation of amorphous Fe into more crystalline forms together with increases in microbial P and decreases in mineral-bound P, but no changes in labile P. Therefore, redox-induced changes in short-term P availability may provide a mechanism by which P from slowly-cycling pools becomes available for biological uptake during succession (Richter et al., 2006).

On the other hand, once dissolved, Fe^{2+} in soil solution can leach into deeper soil layers (Miller et al., 2001), or reprecipitate in (amorphous or) crystalline forms under oxic conditions (Cornell et al., 1989; Cudennec & Lecerf, 2006). Continuous transformation of amorphous into crystalline Fe-oxides over multiple redox cycles has been reported in lab incubations (Thompson et al., 2006) and in-situ (Vogelsang et al., 2016), and presents the likeliest pathway for oxalate Fe loss in this study. Crystalline Fe-oxides, such as goethite, the mineral preferentially formed from free Fe in solution in acidic soils (Cudennec & Lecerf, 2006), can provide similar P sorption abilities as amorphous Fe (Parfitt, 1989), but are much more stable than the latter (Schwertmann, 1991). Lin et al. (2018) found that the sensitivity of Fe and P to redox changes decreased over repeated cycles and linked it to the increased transformation into more stable forms. Phosphorus

solubilization due to reduction events can even happen in anoxic soil microsites under oxic conditions (Liptzin & Silver, 2009). Lower reactivity of P associated with crystalline Fe-oxides may explain why gross P mobilization decreased with succession while abiotic P immobilization, a measure for the P sorption strength of soils, didn't change. It has to be noted at this point that the reported fluxes were measured on rewetted soils that exhibited markedly decreased levels of microbial biomass and phosphomonoesterase activity (Supp. Fig. S2), particularly in late successional forests and OGF, and thus the measured total P mobilization fluxes may not accurately have captured the contribution of biological P mineralization. However, this doesn't weaken the argument made, since effects of Fe-oxides should primarily act on abiotic sorption fluxes. The Fe-P data therefore suggest that Fe reduction events in secondary forests that host higher microbial activity and soil water content cause Fe-oxide dissolution, causing decreases of total P relative to pastures that receive infrequent additions of P fertilizer. Solubilized P can be taken up by plants or microbes while Fe gradually reprecipitates into crystalline forms of higher stability or leach into subsoil. Over time P may increasingly exist in less soluble forms associated with crystalline Fe-oxides, ultimately leading to lower fluxes of P in late-successional forests. Due to a lack of total or dithionite extractable Fe data on topsoils and of oxalate and dithionite Fe fractions in subsoils we cannot disentangle the exact mechanism of Fe-oxide loss over time.

Soil microbial biomass and its C:N:P stoichiometry

Secondary succession at Agua Salud was characterized by a steady increase in microbial P, ultimately leading to a major redistribution of P into microbial biomass and hence microbial immobilization with a higher contribution of microbial biomass P to total P in late successional forests and OGF (increase from 6 to 12% of soil total P). This, however, was not accompanied by similar increases in microbial biomass C and N, which only increased slightly early in succession, so that microbial C:P and N:P ratios decreased steadily. Soil microbial communities usually exhibit homeostatic behaviour towards cellular C and N, but are more flexible in regards to P, which can be stored as inorganic polyphosphates in cells especially under conditions of excess P in soil solution (Albi & Serrano, 2016; Cleveland & Liptzin, 2007; Xu et al., 2013). Similarly, we found a strong

relationship between microbial biomass C and N (Fig. 12a), but a much weaker for microbial biomass C and P (Fig. 12d), as well as for microbial biomass N and P (Fig. 12b). In general, microbial C:P and N:P ratios were very low compared to a OGF study in Central Panama using similar methodology (Turner & Wright, 2014), as well as to global means (Xu et al., 2013). This suggests that microbes took up and stored P in excess compared to C and N, a surprising finding given that total and resin P contents were very low and decreased with successional age in the studied soils, and that other studies found widespread P limitation of microbial processes (Camenzind et al., 2018; Cleveland et al., 2002; Feng & Zhu, 2019) and of microbial biomass (Fanin et al., 2015; Liu et al., 2012; Turner & Wright, 2014) in lowland tropical forests.

The fact that microbial biomass P steadily increased in these severely P-depleted soils during secondary succession without a major response of microbial biomass C has the following important implications: i) Single snapshot measurements of labile P by for instance resin P or Olsen P may not accurately depict the long-term P availability for microbes, ii) P availability for and/or the P demand of microbes increased in secondary forests, and iii) P was not the sole limiting factor for microbial biomass growth in this study. He & Zhu (1998) showed that microbes can access P from mineral-associated pools usually considered as less readily available for uptake. Furthermore, P availability in the tropics can change over short timescales primarily due to the increased influx of C in topsoils during rainfall events (Cleveland et al., 2006; Cleveland & Townsend, 2006; Schwendenmann & Veldkamp, 2005) that may drive redox-induced solubilization of P (Chacon et al., 2006; Lin et al., 2018; Liptzin & Silver, 2009; Peretyazhko & Sposito, 2005), competitive desorption of P from mineral binding sites (Schreeg et al., 2013) and mineralization of P due to priming (Nottingham et al., 2012; Sayer et al., 2011). Such pulse events are hypothesized to be particularly important in the tropics (Lodge et al., 1994), and can lead to microbial immobilization and storage of P at times of elevated short-term P availability (Albi & Serrano, 2016; Olander & Vitousek, 2004; Soong et al., 2018; Yokoyama et al., 2017). During succession litterfall rates quickly reach primary forest levels after 10-20 years of forest recovery (Brown & Lugo, 1990). The build-up of a litter layer may increase C fluxes into soils (Cleveland et al., 2006; Cleveland & Townsend, 2006; Schwendenmann & Veldkamp, 2005), and favour the movement of P from soils into microbial biomass, due to increased P availability during more intense

pulse events. At the same time, labile C inputs can strongly increase microbial activity and hence promote microbial P demand and immobilization potential (Esberg et al., 2010; Olander & Vitousek, 2005; Olander & Vitousek, 2004).

Previous studies in the tropics found that microbial biomass C increased in response to long-term P fertilization (Liu et al., 2012; Turner & Wright, 2014), and that P addition led to a community-wide expression of genes related to microbial biomass growth (Johnston et al., 2019). However, in this study microbial biomass P increased independently from microbial biomass C, indicating that other factors constrained microbial biomass formation and accumulation over time. In support of this, other studies on severely P-impoverished soils found that, without a simultaneous C addition, P fertilization led to a weaker increase of microbial biomass C (Fanin et al., 2015), or that microbial biomass P increased even without any response of microbial biomass C (Soong et al., 2018). Carbon availability is often a limiting factor for soil microbial biomass growth (Rosinger et al., 2019), and a recent study found that increases in microbial biomass during subtropical secondary succession were related to soil organic C accumulation in the rhizosphere (Zheng et al., 2019). The soils in the study are were relatively low in soil organic C (3-4% of dry mass) and in microbial biomass compared to other soils in Central Panama (Turner & Engelbrecht, 2011; Turner & Wright, 2014) and to global estimates (Xu et al., 2013), but were in the range of studies in Amazonia that found co-limitation of microbial biomass growth by C and P (Fanin et al., 2015; Soong et al., 2018). This indicates that the weak increase of microbial biomass C early in succession was due to a more general improvement of abiotic conditions for microbial growth in closed secondary forests compared to open pastures, such as increased soil moisture and re-establishment of litterfall (Brown & Lugo, 1990; Lebrija-Trejos et al., 2011; Lohbeck et al., 2015), but that microbes remained co-limited by C and P throughout the chronosequence.

Although soil organic C is often a good predictor of microbial biomass C (Cleveland & Liptzin, 2007), they were only weakly related in our study (Fig. 12e). However, soil organic C rather reflects the long-term availability and may not fully depict the short-term availability of C for microbes. Rather, it seems like labile C fluxes during litter leaching events are an important source of C for microbes in the tropics, supported by the observation that rainfall can move substantial amounts of dissolved organic C from litter to soils (Cleveland et al., 2006; Schwendenmann & Veldkamp, 2005), leading to

peaks of soil respiration, particularly after periods of high litterfall (Cleveland & Townsend, 2006). Similarly, a litter manipulation experiment in Panama found that litter addition increased annual cumulative soil respiration without changes in soil organic C or microbial biomass C (Sayer et al., 2007, 2011, 2012). Therefore, with the establishment of litterfall during succession, pulse events can temporarily increase C and P availability and overall microbial activity, but are too short-lived to cause a general biomass growth response in these soils low in soil organic C and total P. Microbial P demand and limitation may be particularly high during such periods of high C availability, as indicated in a study by Cleveland et al. (2002) where P addition to a tropical soil only affected soil respiration shortly after addition, but strongly increased the response to labile C additions when added simultaneously. At the same time, Johnston et al. (2019) found that the abundance of polyphosphate accumulating bacteria as well as of polyphosphatase genes were higher in a tropical soil that didn't show a respiration response to P addition compared to P-responsive soils and that P-addition reduced the expression of genes involved in ribosome synthesis and recycling. Accumulation of P-rich ribosomes and polyphosphate whenever P is available in excess over C could help microbes under stationary growth conditions in nutrient-poor systems to better react to fluctuating growth conditions when labile C sources suddenly become available but P is overall scarce (Barnard et al., 2013; Johnston et al., 2019). Therefore, increased storage of P in microbial biomass and resultant low but highly variable microbial C:P and N:P ratios could be a nutrient conservation mechanism in P-limited systems where pulse events are important sources of P (Lodge et al., 1994), and should thus not be interpreted as alleviation of microbial P limitation during succession in our study (Xu et al., 2013), but rather as an increased responsiveness of microbes to infrequent conditions for optimal growth.

Extracellular enzyme activities

Production of extracellular enzymes, which trigger the release of available C and nutrients from polymeric soil organic matter, is thought to be driven by microbial (plant) demand for the respective element and can thus provide additional insight into microbial (plant) nutrient limitation (Marklein & Houlton, 2012; Sinsabaugh et al., 2008;

Waring et al., 2014). In the present study, phosphomonoesterase and phosphodiesterase activities were highly correlated (Supp. Table S1), both increasing 2- to 3-fold during succession. In contrast, enzymes involved in C and N cycling decreased slightly or showed no change, respectively, causing a steady decrease in enzyme C:P and N:P activity ratios. The increase in phosphatase activity was related to decreases in various P pools (e.g. resin P) and increases in soil total and dissolved C:P ratios (Fig. 12c,f and Supp. Table S1). Many studies confirmed that phosphatase activity in the tropics is strongly driven by P availability, both in response to P fertilization (Marklein & Houlton, 2012; Olander & Vitousek, 2000; Turner & Wright, 2014; Yokoyama et al., 2017), and along natural P gradients (Nottingham et al., 2015; Turner et al., 2018). Fewer studies have measured enzyme activities in response to tropical land-use change. For example, Tischer et al. (2014) observed highest phosphatase activities in OGF which had wider soil C:P ratios compared to pastures of different age. The increase of soil C:P ratios during succession, as observed in Tischer et al. (2014) and in this study, may result from lower plant uptake of P relative to C fixation, but also from wider C:P ratios of litter inputs from late-successional trees (Sullivan et al., 2019), and may ultimately require increased investment in phosphatase production in order to get similar returns of P from organic matter. Other studies found no clear trends in phosphatase activities during tropical succession (Acosta-Martínez et al., 2007; Smith et al., 2015), most likely because P availability didn't change consistently.

On the other hand, in this study overall phosphatase activities were in the higher range for Panamanian soils (Turner et al., 2018), and showed steady increases accompanied by decreases in P availability, indicating aggravation of P limitation. Phosphatase enzymes are produced by plant roots (Duff et al., 1994; Tarafdar & Claassen, 1988) and microbes (Nakas et al., 1987), and they both upregulate their production in response to P scarcity (Cabugao et al., 2017; Ma et al., 2019; Ushio et al., 2015; Yokoyama et al., 2017). Since the relative contribution of microbes and plants to phosphatase activity is difficult to disentangle, one should be careful with drawing conclusions as to who was limited by P. In the study area the initially high symbiotic N fixation rates and the relative growth advantage of N fixing plants decreased with progressing succession (Batterman et al., 2013), coinciding with an increase in soil NO_3^- concentrations starting after 30 years of regrowth (this study). This suggests that P became an increasing constraint for plant

growth during succession and makes it likely that plants increased phosphatase production. Microbes however, with increasing biomass P storage and decreasing biomass C:P and N:P ratios, are less likely to become increasingly constrained by P but rather by labile C. In contrast, the redistribution of labile P into soil microbes might have actually promoted plant P limitation. It is therefore uncertain whether and to what extent soil microbial communities may also have upregulated extracellular phosphatase production. Carbon acquisition enzymes such as ß-glucosidase decreased slightly, and it has been speculated that ß-glucosidase is produced constitutively during microbial growth, rather than being driven by C demand (Rosinger et al., 2019). However, microbial P demand and phosphatase production may be especially high during pulse labile C inputs when microbes start mining for P (Nottingham et al., 2012). Since extracellular enzymes have long turnover times (Schimel et al., 2017), the enzymes produced during periods of peak microbial activity can thus remain stable and active for extended periods of time.

Implications for P cycling during tropical secondary succession

In this study we reported a new mechanism by which P limitation aggravates during tropical secondary succession, caused by the redistribution of P from soils into microbes, which in late successional forests and OGF held 10-15% of soil total P. This mechanism likely differs from other successional studies in that it may primarily act in particularly C and P impoverished soils, indicated by low soil total P, low soil organic C and low microbial biomass C as found here. Under such conditions, microbial activity in bulk soil relies on pulse C additions that are re-introduced when litterfall rates recuperate and roots proliferate in secondary forests (Brown & Lugo, 1990; Cleveland & Townsend, 2006; Sayer et al., 2011). In low-P soils microbes are particularly strong competitors for P when C inputs increase (Esberg et al., 2010; Olander & Vitousek, 2005). Immobilization and intracellular storage of P under fluctuating growth conditions may benefit microbes when sudden conditions of high P demand arise (Albi & Serrano, 2016; Cleveland et al., 2002; Johnston et al., 2019). We suggest that particularly P solubilization via amorphous Fe-oxide reduction is a viable P source for microbes in secondary forests. As succession progresses, P in topsoil is continuously removed by biological uptake and at the same

time becomes increasingly associated with more stable crystalline Fe oxide forms, leading to lower diffusion fluxes and mobilization rates of inorganic P later in succession. In response, plants (and microbes) increase phosphatase production to access P from organic matter, indicating that P cycling becomes more biologically controlled during succession. The changes in soil P conditions could potentially affect the forests' future C sink strength (Fleischer et al., 2019), or drive the compositional turnover of plants towards a late-successional community highly specialized on low-P conditions (Condit et al., 2013; Sullivan et al., 2019; Turner et al., 2018; van Breugel et al., 2019; Wright et al., 2018). Likewise, late-successional forests could host a microbial community specifically adapted to exploit organic P sources (Yao et al., 2018), and to efficiently immobilize and store P in order to rapidly respond to fluctuating growth conditions (Johnston et al., 2019). Since P becomes increasingly locked up in microbial biomass late in succession, increased belowground C allocation of plants may increase microbial activity in the rhizosphere and help keeping P in an actively cycling pool (Spohn & Kuzyakov, 2013). Taken together, our results support that, similarly to what Davidson et al. (2007) suggested, processes during long-term ecosystem development can be mirrored in patterns over relatively short-term secondary successions. With the redistribution of P into microbial biomass, cycling of P and the competition between plants and microbes likely becomes tighter, and the importance of organic P as P source increases.

5. Figures

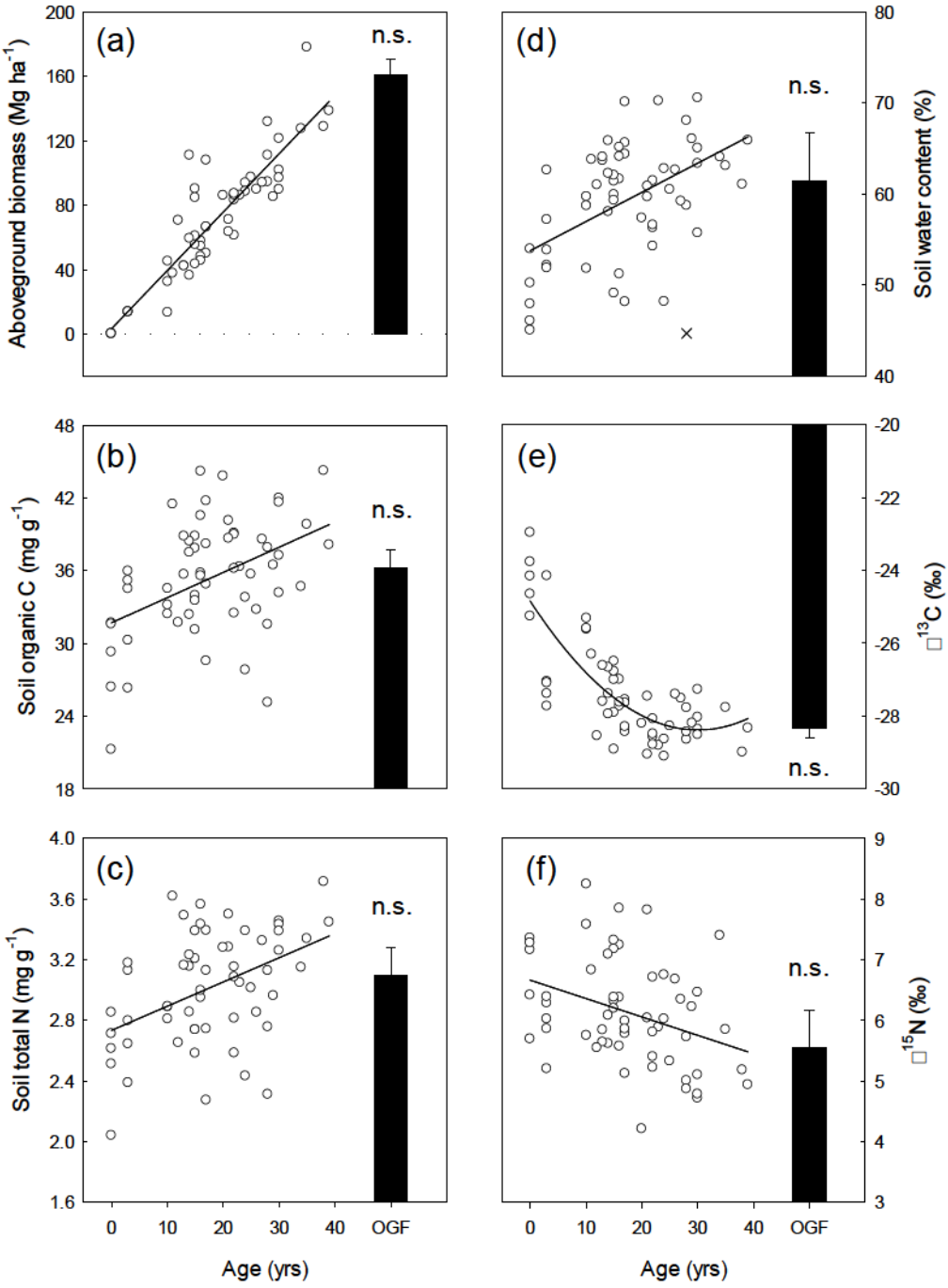


Figure 1. Effect of time since pasture abandonment (0 yrs – pastures, up to 39 yrs old secondary forests) on different ecosystem (vegetation and soil) properties in secondary forests compared to old-growth forests (OGF) in the Agua Salud watershed, Panama. Regression analysis shows the relationship between secondary forest age and (a) aboveground biomass (R^2=0.83; p<0.001; n=58; y=3.624x+3.372), (b) soil organic C (R^2=0.19; p<0.001; n=58; y=0.2079x+31.7019), (c) soil total N (R^2=0.19; p<0.001; n=58; y=0.0160x+2.7327), (d) soil water content (R^2=0.27; p<0.001; n=57; y=0.321x+53.7773), (e) soil $\delta^{13}C$ values (R^2=0.62; p<0.001; n=58; $y=0.0039x^2-0.2363x-24.8367$) and (f) soil $\delta^{15}N$ values (R^2=0.12; p=0.007; n=58; y=-0.0303x+6.6643). Outliers are indicated as crosses and were not included in regression analyses. Bar-charts represent the mean value ± 1 standard error of 5 OGF of unknown age. Symbols above the bars

indicate significant differences between OGF and the 5 oldest secondary forests, tested by Student's t-tests or Mann-Whitney U-tests (°, p<0.1, *, p<0.05, **, p<0.01, ***, p<0.001, n.s.=p>0.05). Mann-Whitney U-test was used in Fig. 1(b).

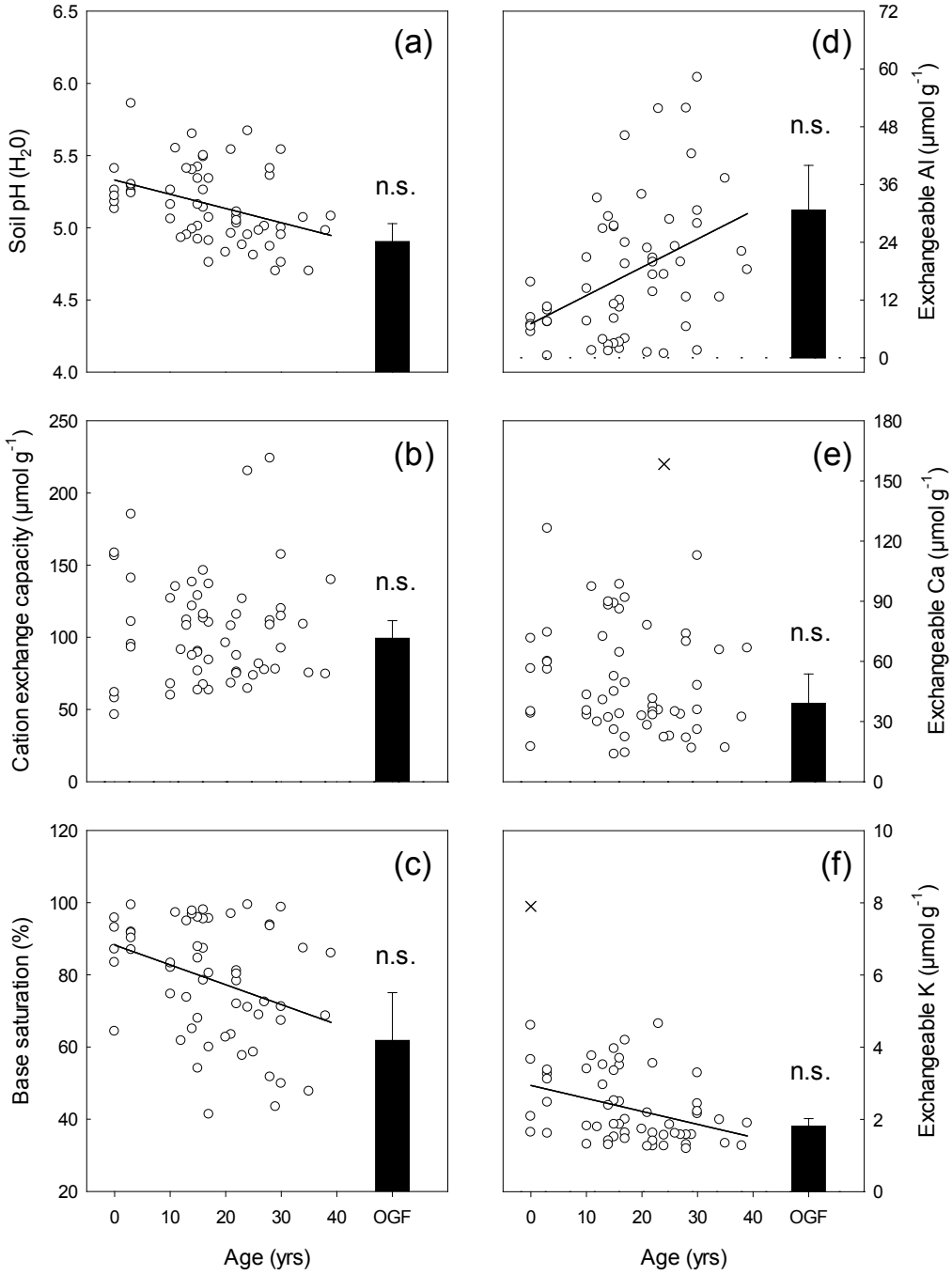


Figure 2. Effect of time since pasture abandonment (0 yrs – pastures, up to 39 yrs old secondary forests) on soil physicochemical properties in secondary forests compared to old-growth forests (OGF) in the Agua Salud watershed, Panama. Regression analysis shows the relationship between secondary forest age and (a) soil pH (R^2=0.15; p=0.003; n=58; y=-0.0098x+5.3308), (b) cation exchange capacity (R^2<0.01; p=0.656; n=58), (c) base saturation (R^2=0.12; p=0.008; n=58; y=-0.5497x+88.2628), (d) exchangeable Al (R^2=0.16; p=0.002; n=58; sqrt(y)=0.0695x+2.5685), (e) exchangeable Ca (R^2=0.03; p=0.193; n=57) and (f) exchangeable K (R^2=0.14; p=0.005; n=57; y=-0.0359x+2.9422). Outliers are indicated as crosses and were not included in regression analyses. Bar-charts represent the mean value ± 1 standard error of 5 OGF of

unknown age. Symbols above the bars indicate significant differences between OGF and the 5 oldest secondary forests, tested by Student's t-tests or Mann-Whitney U-tests (◌•, p<0.1, *, p<0.05, **, p<0.01, ***, p<0.001, n.s.=p>0.05). Mann-Whitney U-test was used in Fig. 2(d).

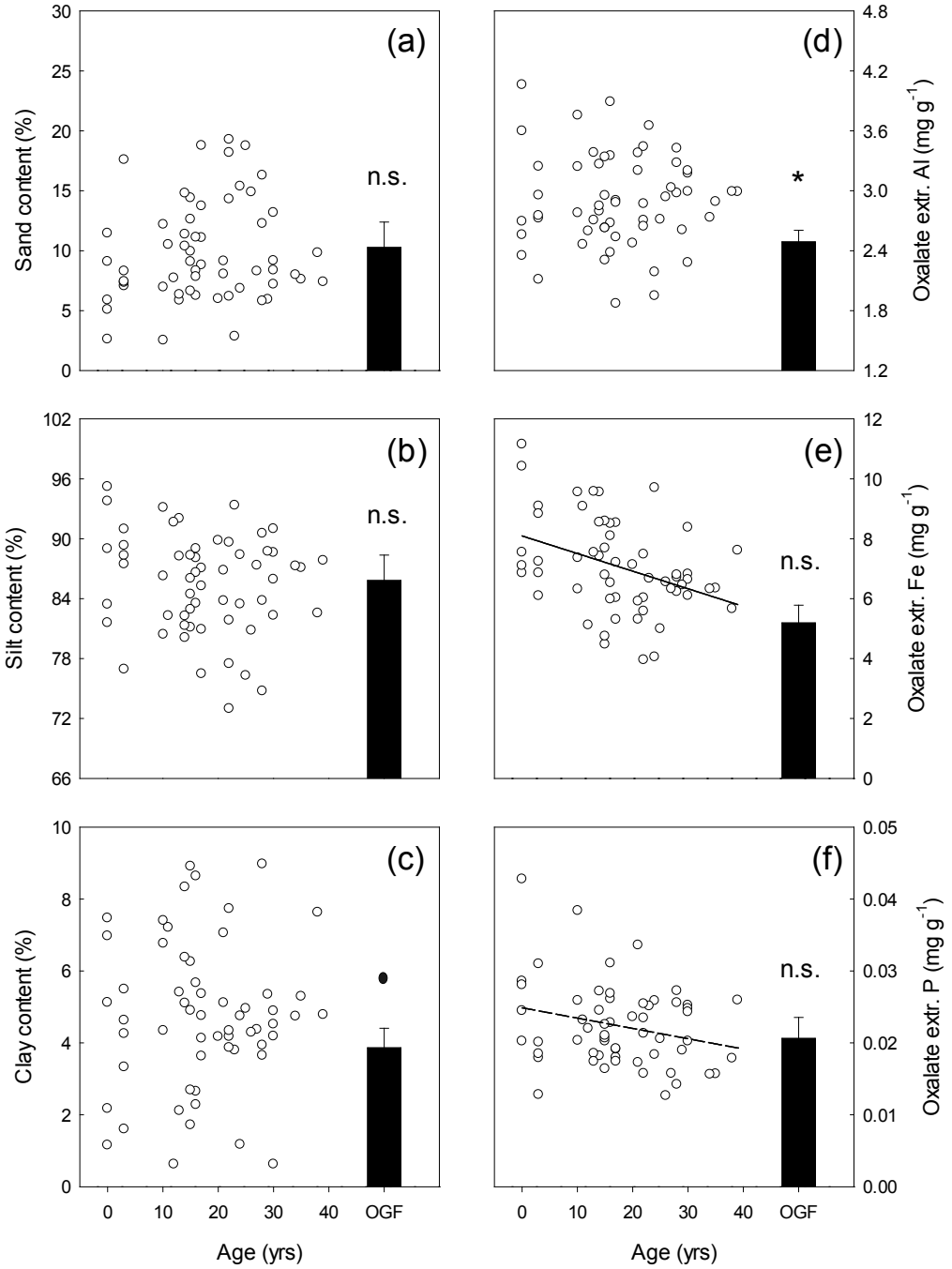


Figure 3. Effect of time since pasture abandonment (0 yrs – pastures, up to 39 yrs old secondary forests) on soil physicochemical properties in secondary forests compared to old-growth forests (OGF) in the Agua Salud watershed, Panama. Regression analysis shows the relationship between secondary forest age and (a) sand content (R^2=0.02; p=0.267; n=58), (b) silt content (R^2=0.02; p=0.279; n=58), (c) clay content (R^2<0.01; p=0.702; n=58), (d) oxalate extractable Al (R^2<0.01; p=0.926; n=58), (e) oxalate extractable Fe (R^2=0.15; p=0.003; n=58; y=-0.0588x+8.0960) and (f) oxalate extractable P (R^2=0.06; p=0.057; n=58; y=-0.0001x+0.0249). Bar-charts represent the mean value ± 1 standard error of 5 OGF of unknown age.

Symbols above the bars indicate significant differences between OGF and the 5 oldest secondary forests, tested by Student's t-tests or Mann-Whitney U-tests (•, p<0.1, *, p<0.05, **, p<0.01, ***, p<0.001, n.s.=p>0.05). Mann-Whitney U-tests were used in Fig. 3(a,b,c,f).

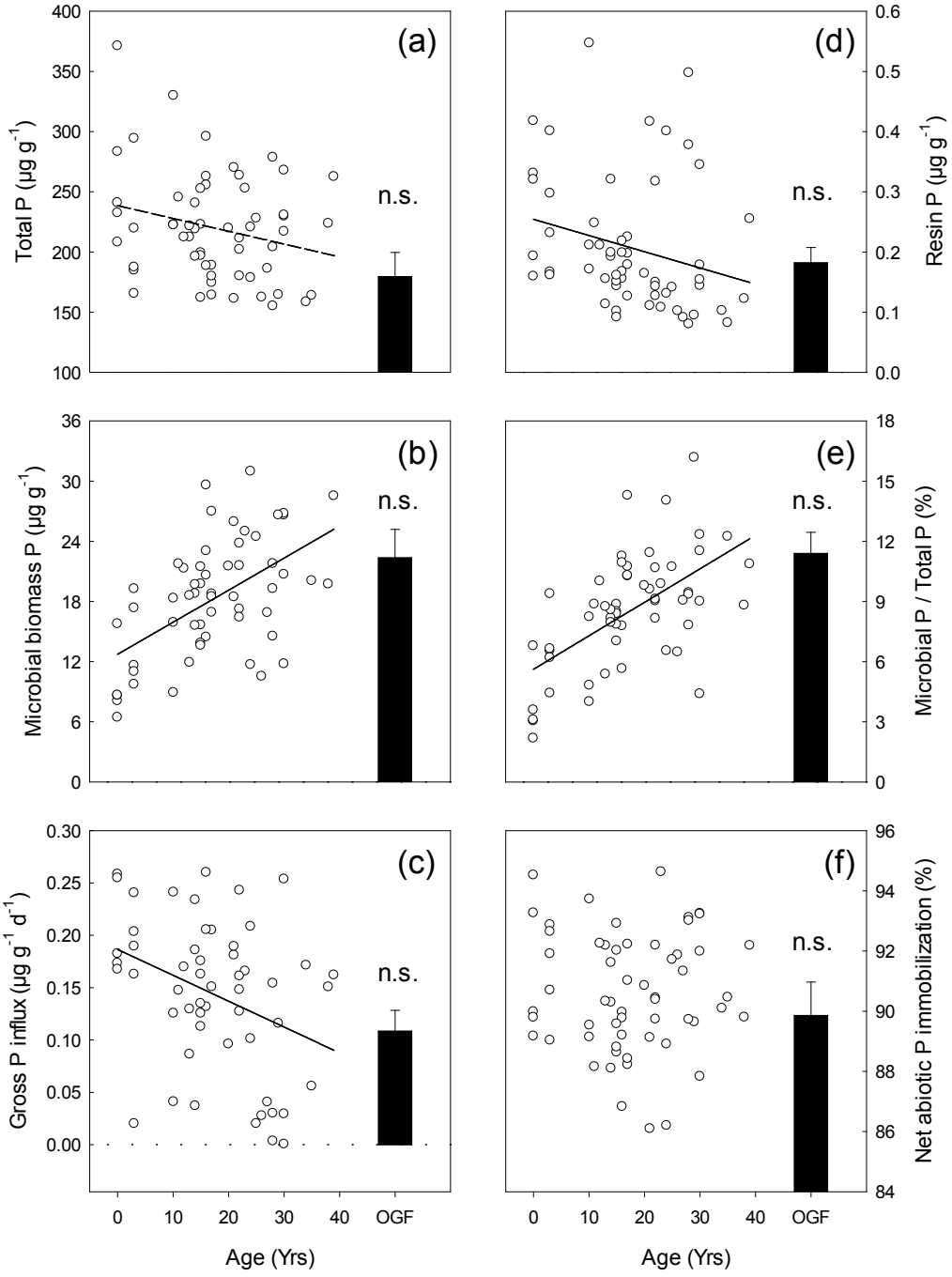


Figure 4. Effect of time since pasture abandonment (0 yrs – pastures, up to 39 yrs old secondary forests) on soil P pools and P fluxes in secondary forests compared to old-growth forests (OGF) in the Agua Salud watershed, Panama. Regression analysis shows the relationship between secondary forest age and (a) total P (R^2=0.06; p=0.067; n=58; y=-1.0647x+238.5466), (b) microbial biomass P (R^2=0.30; p<0.001; n=57; y=0.3203x+12.7160), (c) gross P mobilization (R^2=0.13; p=0.008; n=54; y=-0.0025x+0.1866), (d) resin P (R^2=0.11; p=0.011; n=58; log(y)=-0.0156x-1.4193), (e) microbial biomass P/total P (R^2=0.35; p<0.001; n=57; y=0.1672x+5.6126) and (f) net abiotic P immobilization (R^2<0.01; p=0.836; n=58). Bar-charts represent the

mean value ± 1 standard error of 5 OGF of unknown age. Symbols above the bars indicate significant differences between OGF and the 5 oldest secondary forests, tested by Student's t-tests (•, p<0.1, *, p<0.05, **, p<0.01, ***, p<0.001, n.s.=p>0.05).

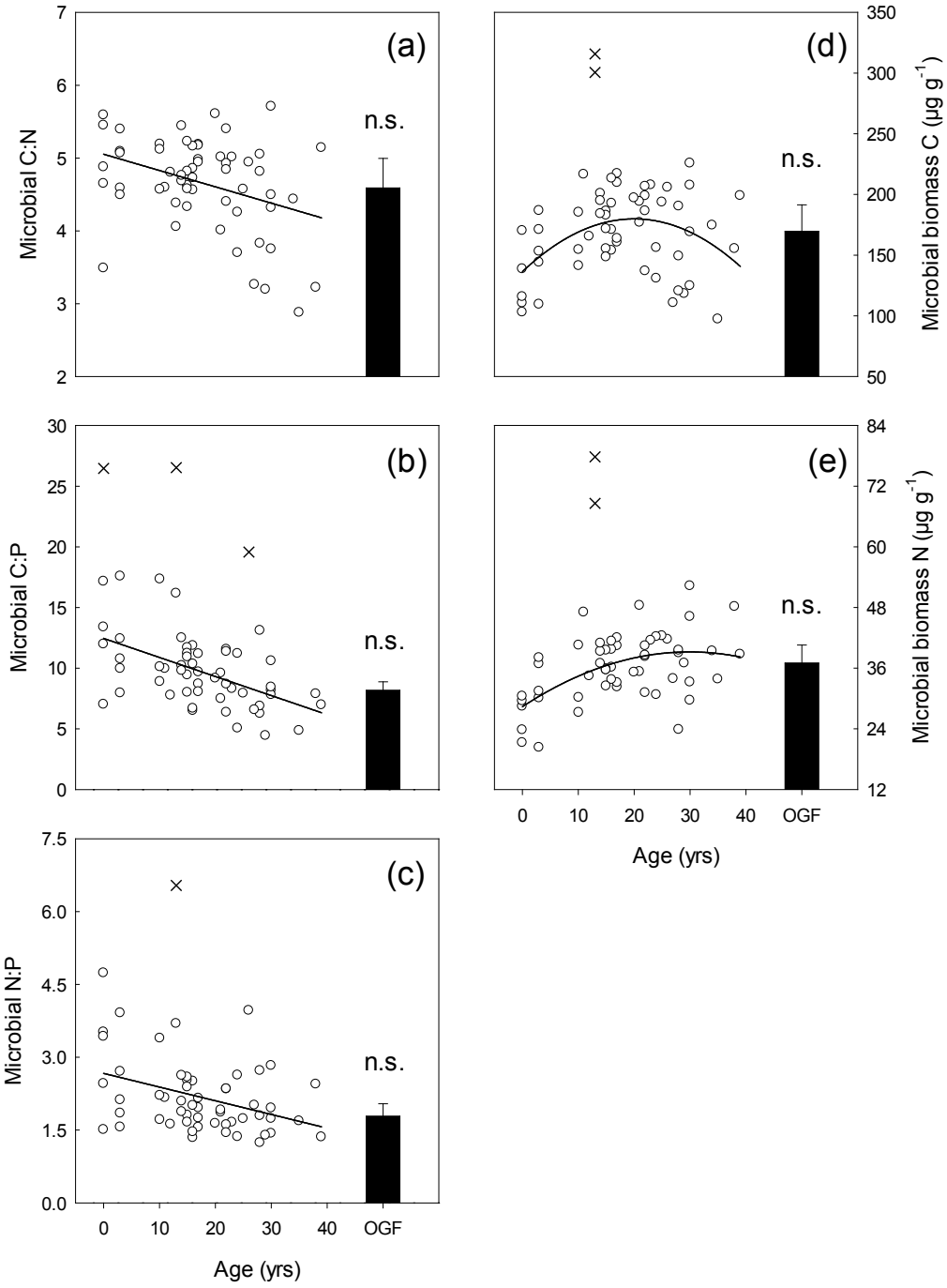


Figure 5. Effect of time since pasture abandonment (0 yrs – pastures, up to 39 yrs old secondary forests) on microbial stoichiometry and microbial biomass in secondary forests compared to old-growth forests (OGF) in the Agua Salud watershed, Panama. Regression analysis shows the relationship between secondary forest age and (a) microbial C:N (R^2=0.13; p=0.006; n=58; y=-0.0224x+5.0538), (b) microbial C:P (R^2=0.27; p<0.001; n=54; y=-0.1570+12.4542), (c) microbial N:P (R^2=0.14; p=0.004; n=56; y=-0.0282x+2.6681), (d) microbial biomass C (R^2=0.19; p=0.004; n=56; y=-0.1087x^2+4.3508x+136.3701) and (e) microbial biomass N (R^2=0.28; p<0.001; n=56; y=-0.0121x^2+0.7244x+28.3721). Outliers are indicated as crosses and were not included in regression analyses. Bar-charts represent the mean value ± 1 standard error of 5 OGF of unknown age. Symbols above the bars indicate significant differences between OGF and

the 5 oldest secondary forests, tested by Student's t-tests (•, p<0.1, *, p<0.05, **, p<0.01, ***, p<0.001, n.s.=p>0.05).

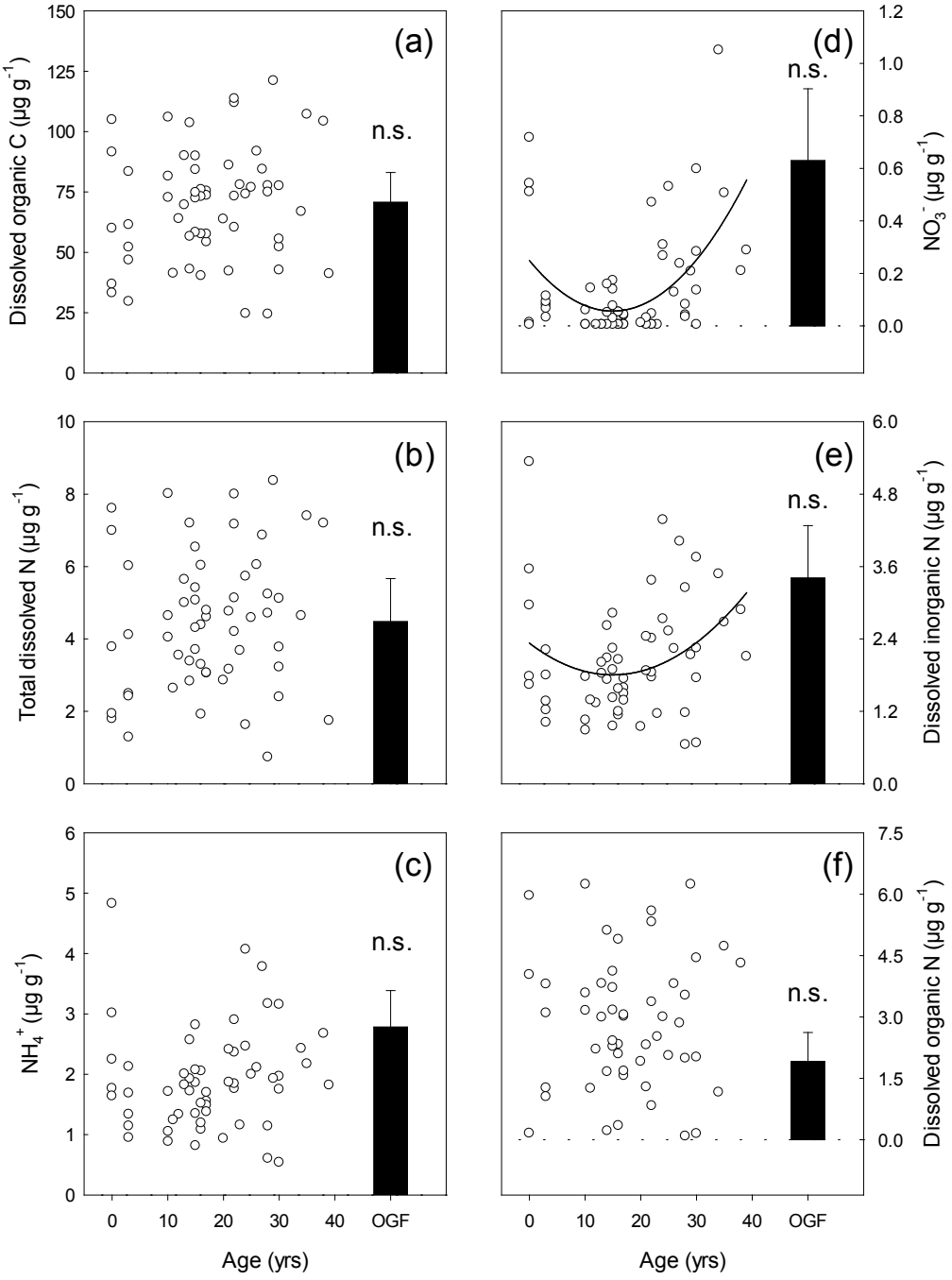


Figure 6. Effect of time since pasture abandonment (0 yrs – pastures, up to 39 yrs old secondary forests) on labile soil C and N pools in secondary forests compared to old-growth forests (OGF) in the Agua Salud watershed, Panama. Regression analysis shows the relationship between secondary forest age and (a) dissolved organic C (R^2=0.02; p=0.267; n=58), (b) total dissolved N (R^2=0.02; p=0.327; n=58), (c) soil ammonium (R^2<0.01; p=0.489; n=58), (d) soil nitrate (R^2=0.28; p<0.001; n=58; y=0.0009x^2-0.0258x+0.2490), (e) dissolved inorganic N (R^2=0.10; p=0.049; n=58; y=-0.0024x^2-0.0703x+2.3295) and (f) dissolved organic N (R^2<0.01; p=0.910; n=52). Bar-charts represent the mean value ± 1 standard error of 5 OGF of unknown age. Symbols above the bars indicate significant differences between OGF and the 5 oldest secondary forests, tested by Student's t-tests or Mann-Whitney U-tests (•, p<0.1, *, p<0.05, **,

p<0.01, ***, p<0.001, n.s.=p>0.05). Mann-Whitney U-tests were used in Fig. 7(c,d).

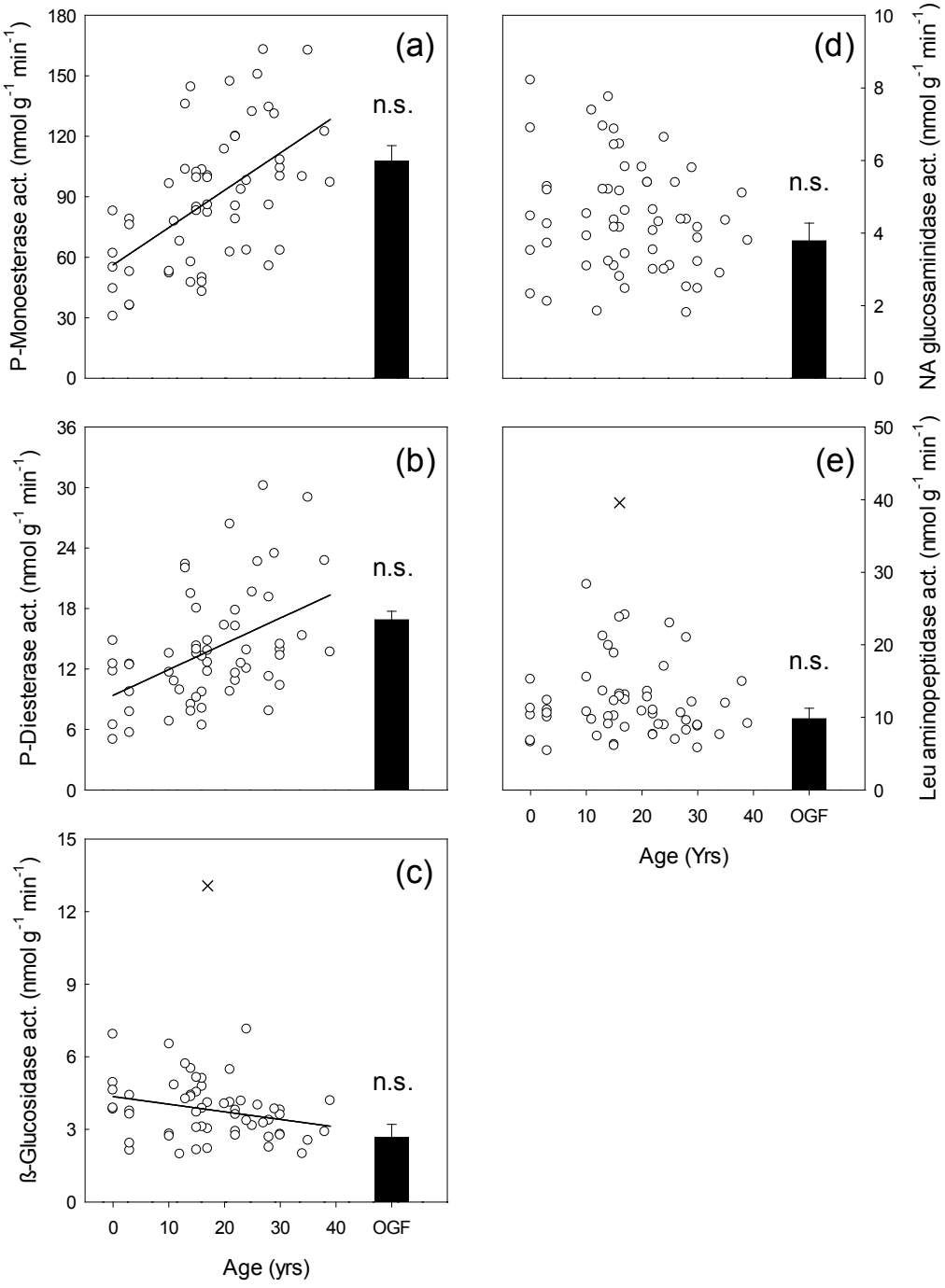


Figure 7. Effect of time since pasture abandonment (0 yrs – pastures, up to 39 yrs old secondary forests) on soil enzyme activities in secondary forests compared to old-growth forests (OGF) in the Agua Salud watershed, Panama. Regression analysis shows the relationship between secondary forest age and (a) phosphomonoesterase activity (R^2=0.32; p<0.001; n=58; y=1.8514x+56.1741), (b) phosphodiesterase activity (R^2=0.21; p<0.001; n=58; y=0.2559x+9.3704), (c) ß-glucosidase activity (R^2=0.07; p=0.041; n=57; y=-0.0314x+4.3568), (d) N-acetyl-ß-D-glucosaminidase activity (R^2=0.04; p=0.160; n=58) and (e) L-Leucine aminopeptidase activity (R^2<0.01; p=0.868; n=57). Outliers are indicated as crosses and were not included in regression analyses. Bar-charts represent the mean value ± 1 standard error of 5 OGF of unknown age. Symbols above the bars indicate significant differences between OGF and the 5 oldest secondary forests, tested by Student's t-tests or Mann-Whitney U-tests (•, p<0.1, *, p<0.05, **, p<0.01, ***, p<0.001,

n.s.=p>0.05). Mann-Whitney U-test was used in Fig. 8(b).

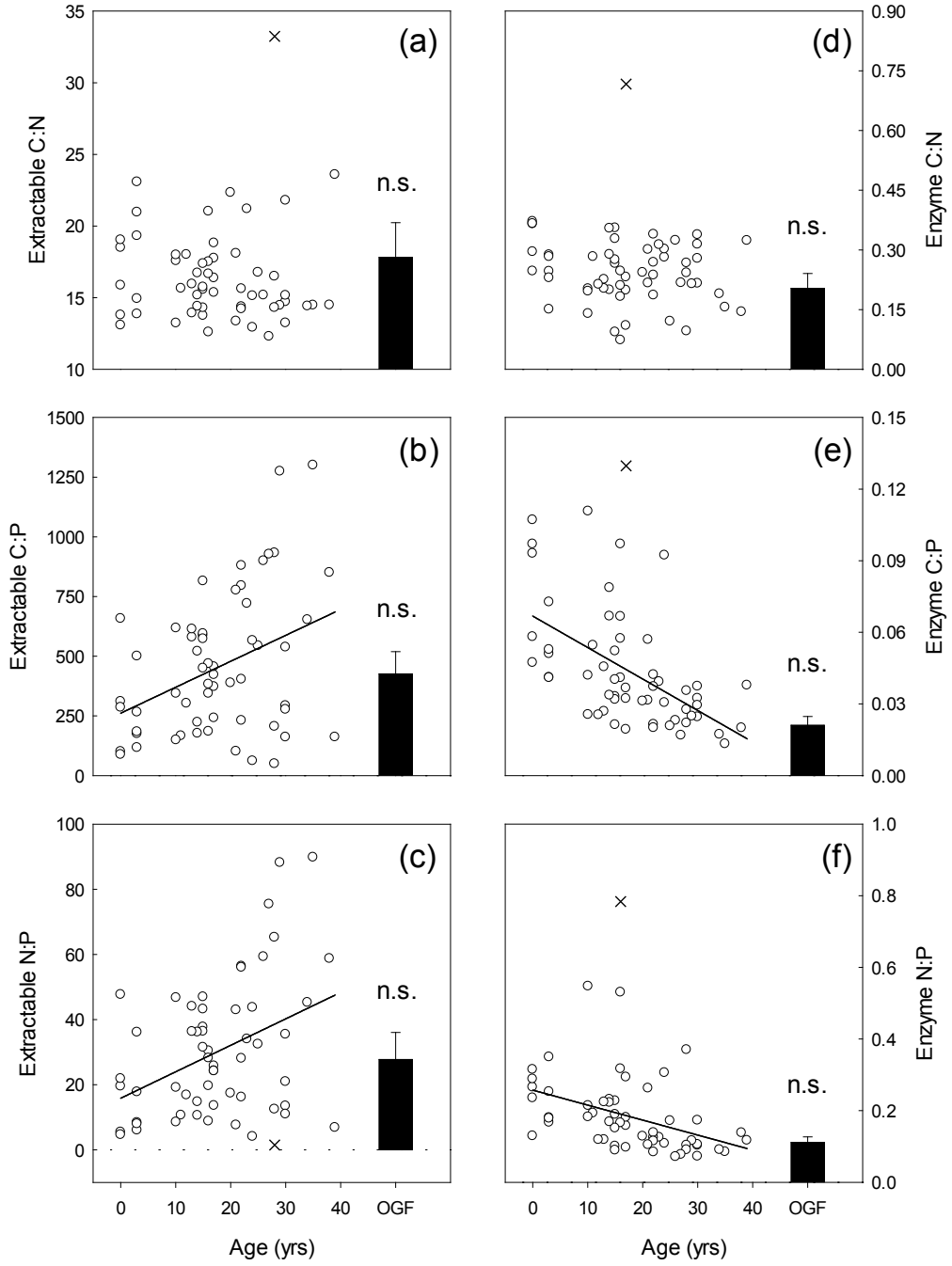


Figure 8. Effect of time since pasture abandonment (0 yrs – pastures, up to 39 yrs old secondary forests on extractable and enzymatic C:N:P stoichiometry in secondary forests compared to old-growth forests (OGF) in the Agua Salud watershed, Panama. Regression analysis shows the relationship between secondary forest age and (a) extractable C:N (R^2=0.01; p=0.447; n=57), (b) extractable C:P (R^2=0.14; p=0.004; n=58; y=10.8638x+261.1528), (c) extractable N:P (R^2=0.16; p=0.002; n=57; y=0.8125x+15.8300), (d) enzyme C:N (R^2=0.03; p=0.219; n=57), (e) enzyme C:P (R^2=0.37; p<0.001; n=57; log(y)=-0.0303x-2.7331) and (f) enzyme N:P (R^2=0.27; p<0.001; n=57; y=-0.0248x-1.3486). Outliers are indicated as crosses and were not included in regression analyses. Bar-charts represent the mean value ± 1 standard error of 5 OGF of unknown age. Symbols above the bars indicate significant differences between OGF and the 5 oldest secondary forests, tested by Student's t-tests or Mann-Whitney U-tests (•, p<0.1, *, p<0.05, **, p<0.01, ***, p<0.001,

n.s.=p>0.05). Mann-Whitney U-tests were used in Fig. 9(a,b,c,f).

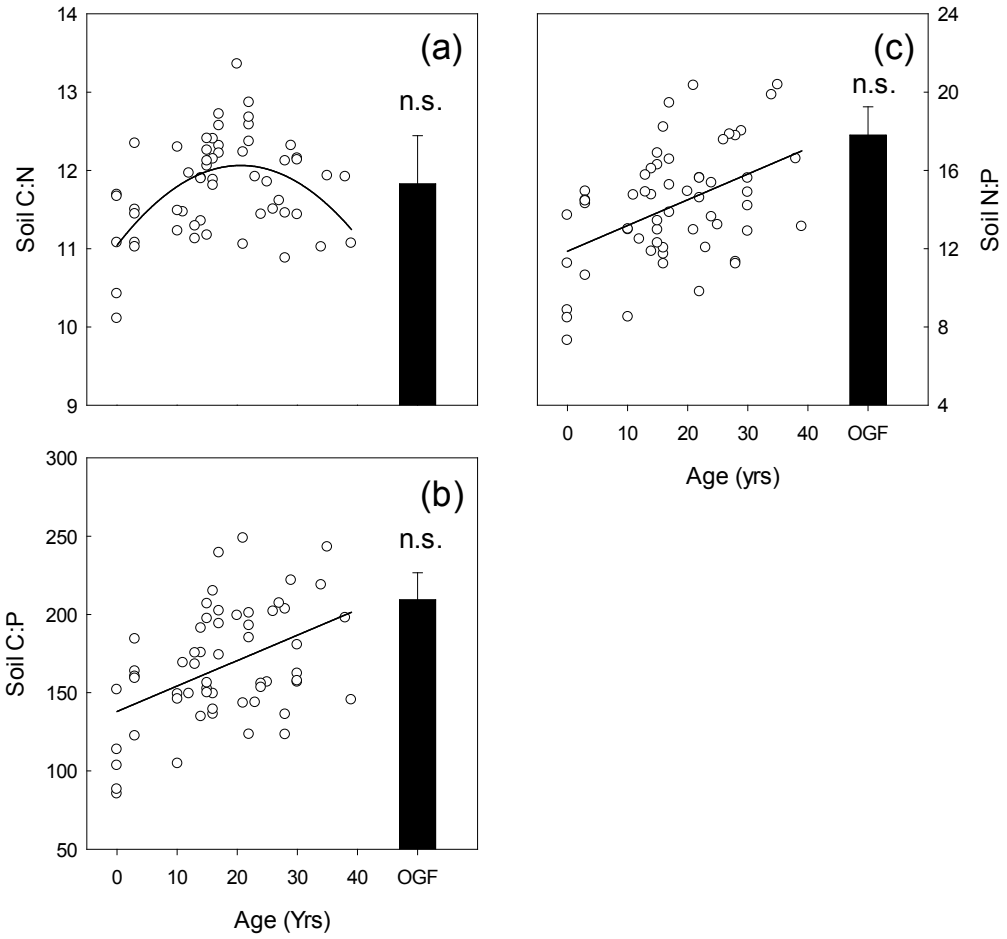


Figure 9. Effect of time since pasture abandonment (0 yrs – pastures, up to 39 yrs old secondary forests) on soil C:N:P stoichiometry in secondary forests compared to old-growth forests (OGF) in the Agua Salud watershed, Panama. Regression analysis shows the relationship between secondary forest age and (a) soil C:N (R^2=0.29; p<0.001; n=56; $y=-0.0024x^2+0.0992x+11.0427$), (b) soil C:P ($R^2$=0.20; p<0.001; n=58; y=1.6285x+137.9412) and (c) soil N:P (R^2=0.21; p<0.001; n=58; y=0.1315x+11.8686). Bar-charts represent the mean value ± 1 standard error of 5 OGF of unknown age. Symbols above the bars indicate significant differences between OGF and the 5 oldest secondary forests, tested by Student's t-tests or Mann-Whitney U-tests (•, p<0.1, *, p<0.05, **, p<0.01, ***, p<0.001, n.s.=p>0.05). Mann-Whitney U-tests were used in Fig. 6(a,c).

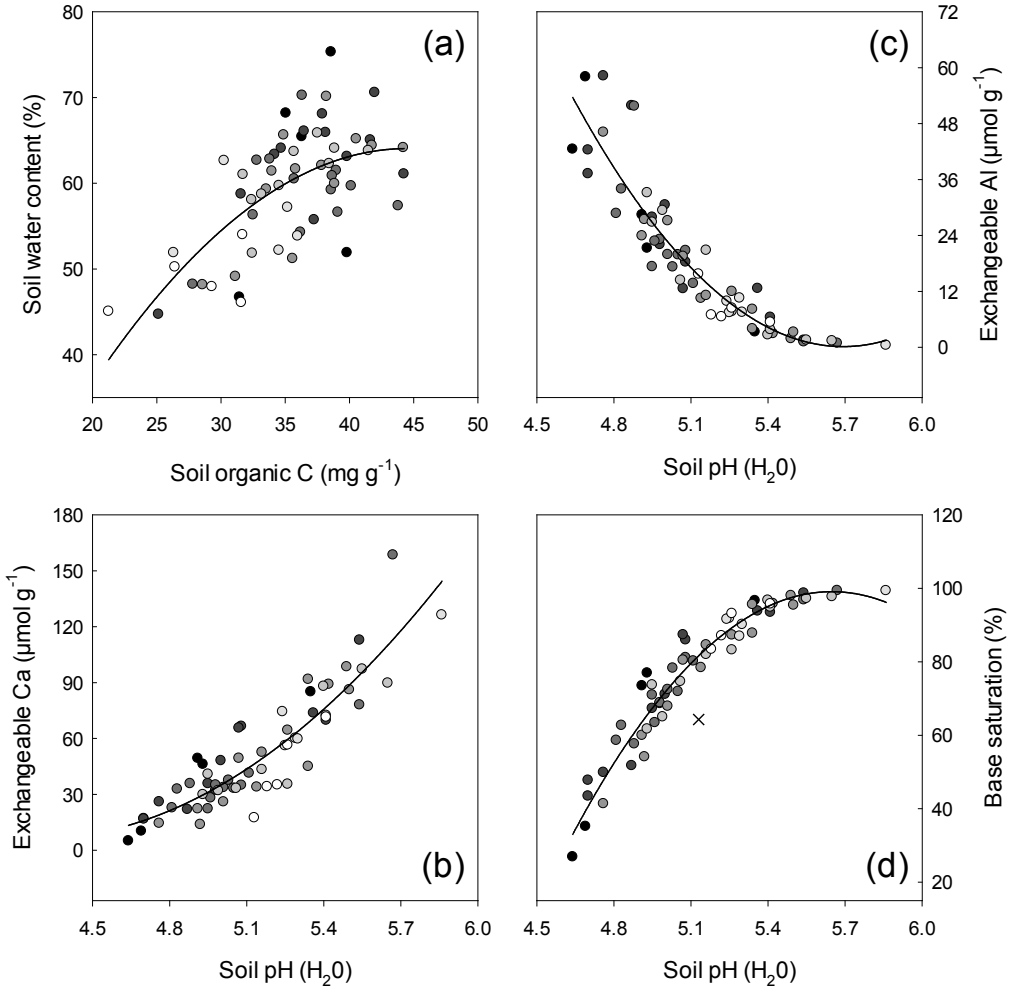


Figure 10. Relationships between individual soil physicochemical properties in pastures, secondary forests and old-growth forests (OGF) in the Agua Salud watershed, Panama. Regression analysis shows the relationship between (a) soil organic C and soil water content (R^2=0.44; p<0.001; n=63), (b) soil pH and exchangeable Ca^{2+} (R^2=0.81; p<0.001; n=63), (c) soil pH and exchangeable Al^{3+} (R^2=0.93; log(p)<0.001; n=63) and (d) soil pH and base saturation (R^2=0.94; p<0.001; n=62). Outliers are indicated as crosses and were not included in regression analyses. Increase from white to grey and black fill colors codes increasing age after pasture abandonment.

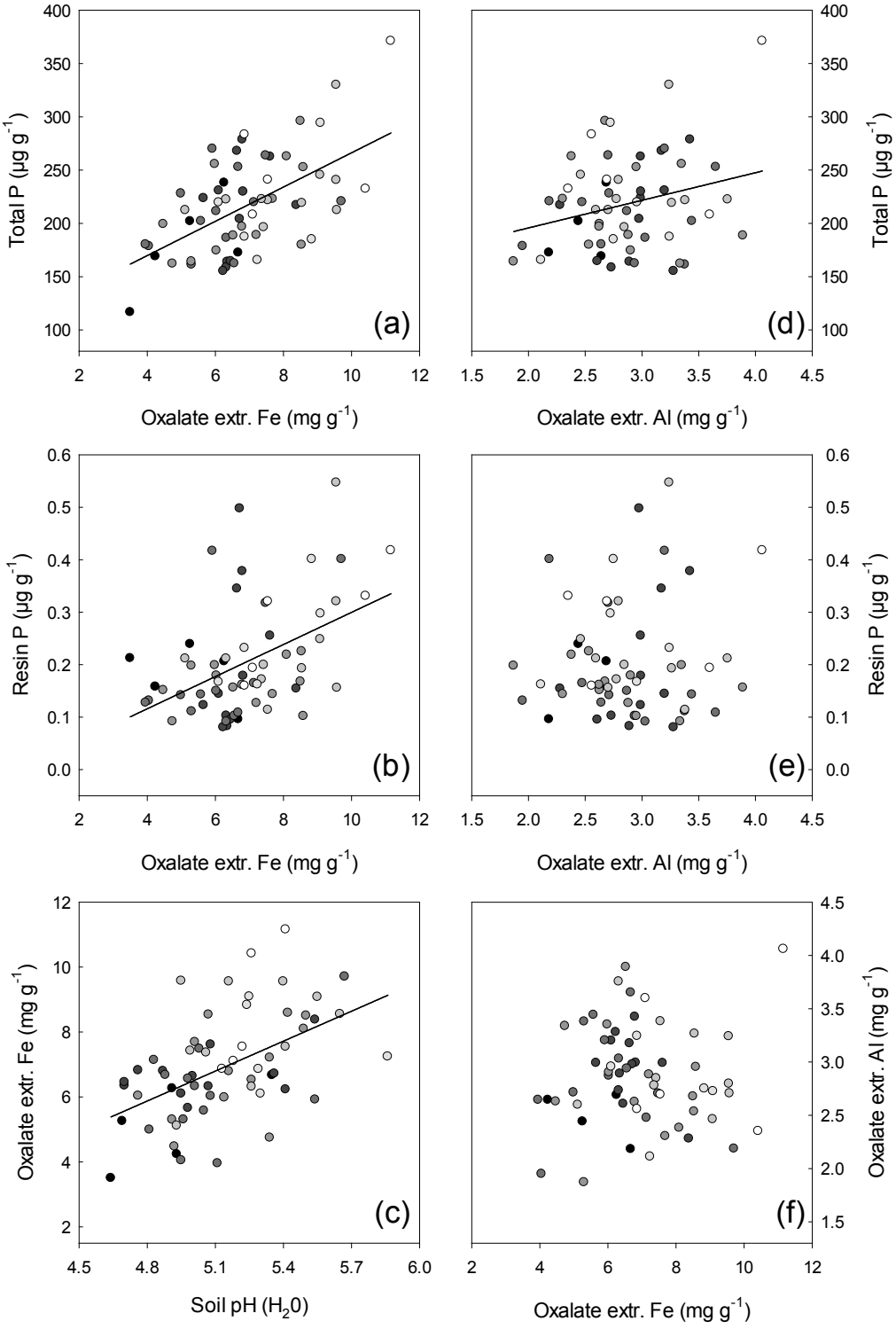


Figure 11. Relationships between individual soil physicochemical properties and P pools in pastures, secondary forests and old-growth forests (OGF) in the Agua Salud watershed, Panama. Regression analysis shows the relationship between (a) oxalate extractable Fe and total P (R^2=0.32; p<0.001; n=63), (b) oxalate extractable Fe and resin P (R^2=0.20; log(p)<0.001; n=63), (c) soil pH and oxalate extractable Fe (R^2=0.26; log(p)<0.001; n=63), (d) oxalate extractable Al and total P (R^2=0.10; p=0.011; n=63), (e) oxalate extractable Al and resin P (R^2<0.01; log(p)=0.875; n=63) and (f) oxalate extractable Fe and oxalate extractable Al (R^2=0.01; p=0.504; n=63). Increase from white to grey and black fill colors codes increasing age after pasture abandonment.

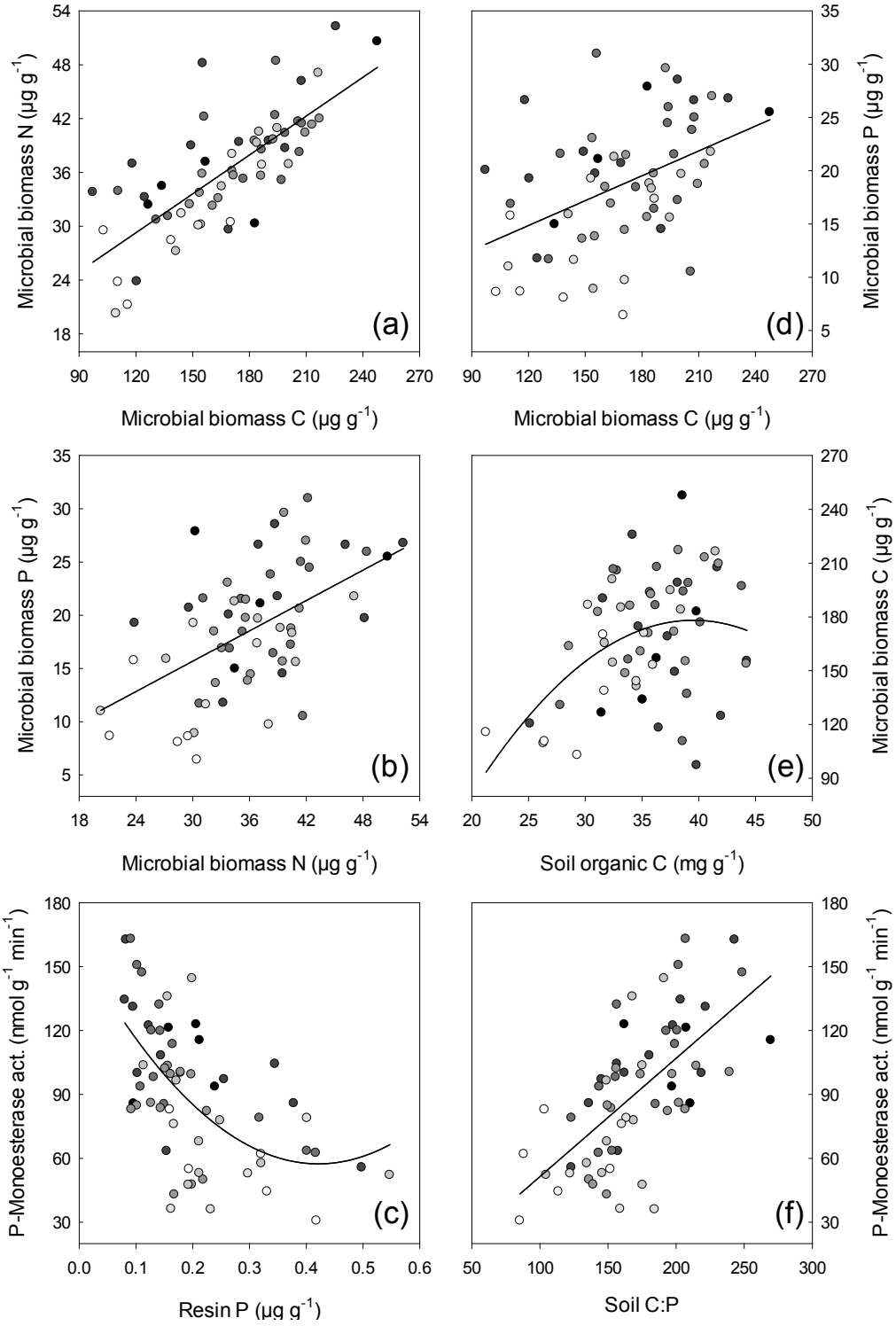
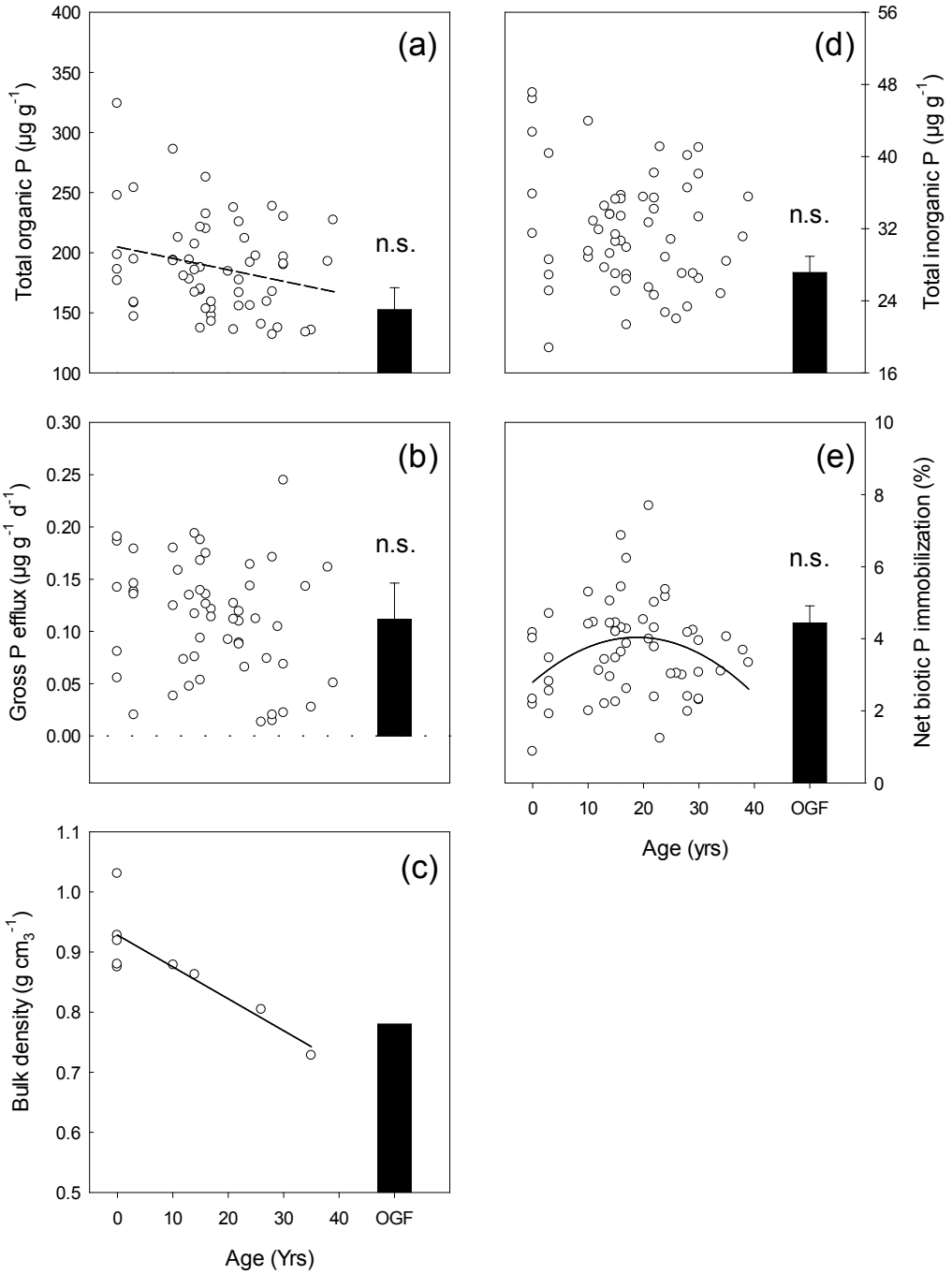


Figure 12. Relationships between microbial biomass C, N and P pools, between soil organic C and microbial biomass C and between phosphomonoesterase activity and resin P and soil C:P in pastures, secondary forests and old-growth forests (OGF) in the Agua Salud watershed, Panama. Regression analysis shows the relationship between (a) microbial biomass C and microbial biomass N (R^2=0.56; p<0.001; n=61), (b) microbial biomass N and microbial biomass P (R^2=0.29; p<0.001; n=61), (c) resin P and phosphomonoesterase activity (R^2=0.26; log(p)<0.001; n=63), (d) microbial biomass C and microbial biomass P (R^2=0.20; p<0.001; n=61), (e) soil organic C and microbial biomass C (R^2=0.19; p=0.002; n=61)

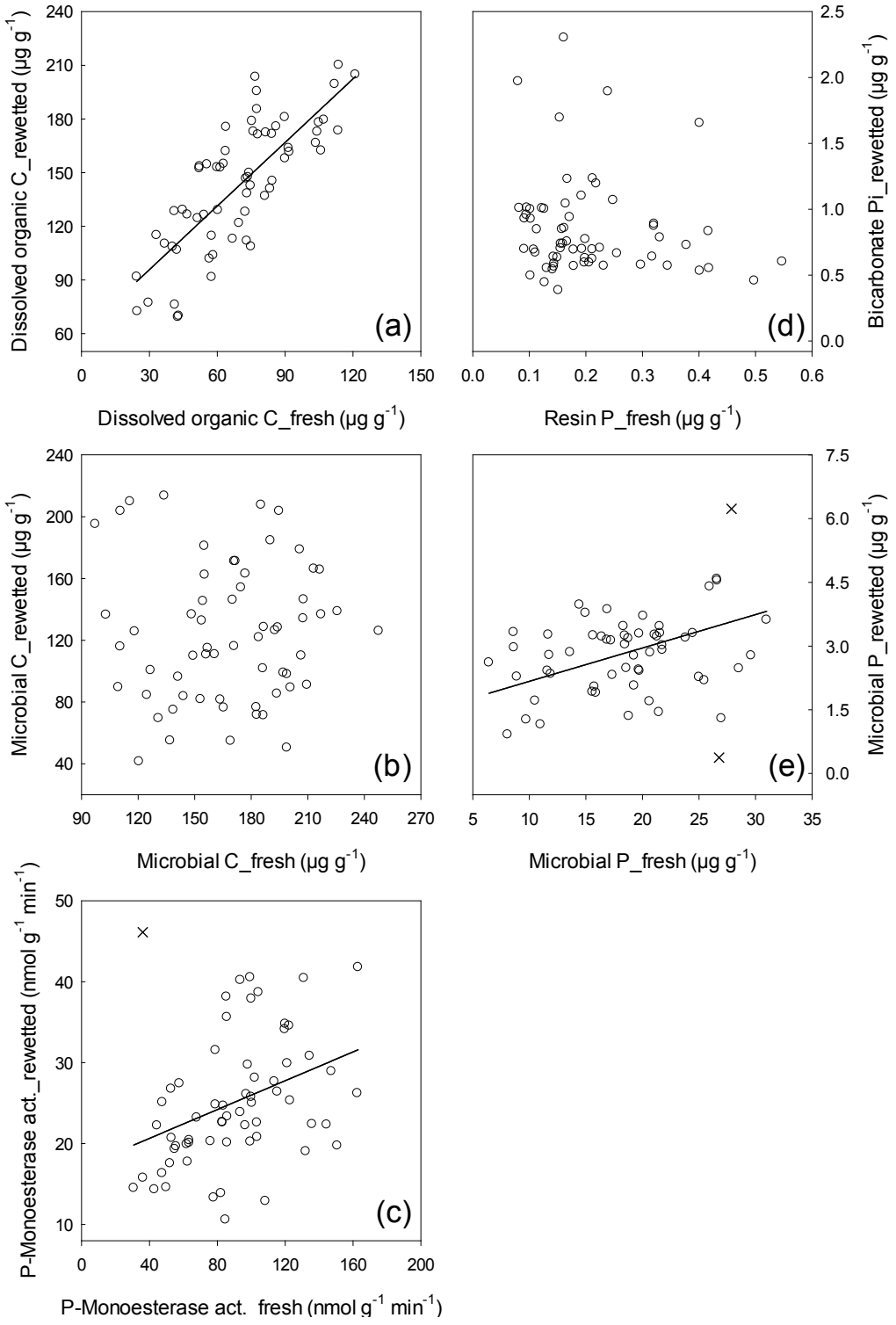
and (f) soil C:P and phosphomonoesterase activity (R^2=0.35; p<0.001; n=63). Increase from white to grey and black fill colors codes increasing age after pasture abandonment.

6. Supplementary figures and tables



Supplementary Figure S1. Effect of time since pasture abandonment (0 yrs – pastures, up to 39 yrs old secondary forests on P pools, P fluxes and bulk density in secondary forests compared to old-growth forests (OGF) in the Agua Salud watershed, Panama. Regression analysis shows the relationship between secondary forest age and (a) total organic P (R^2=0.06; p=0.067; n=58; y=-0.9551x+204.8868), (b) gross P

efflux (R^2=0.05; p=0.101; n=54), (c) bulk density (R^2=0.75; p=0.003; n=9; log(y)=-0.0063x-0.0746), (d) total inorganic P (R^2=0.03; p=0.182; n=58) and (e) net biotic immobilization (R^2=0.11; p=0.040; n=58; $y=0.0035x^2+0.1326x+2.7918$). Bar-charts represent the mean value ± 1 standard error of 5 OGF of unknown age. Symbols above the bars indicate significant differences between OGF and the 5 oldest secondary forests, tested by Student's t-tests or Mann-Whitney U-tests (•, p<0.1, *, p<0.05, **, p<0.01, ***, p<0.001, n.s.=p>0.05). Mann-Whitney U-test was used in Supp. Fig. S1(e). Due to low replication (OGF n=1), differences could not be statistically tested for in Supp. Fig. 1(c).



Supplementary Figure S2. Relationships between soil parameters measured on fresh soils and rewetted soils in pastures, secondary forests and old-growth forests (OGF) in the Agua Salud watershed, Panama. Regression analysis shows the relationship between fresh soil measurements and rewetted soil measurements for (a) dissolved organic C (R^2=0.61; p<0.001; n=63), (b) microbial biomass C (R^2<0.01; p=0.854; n=61), (c) phosphomonoesterase activity (R^2=0.22; p<0.001; n=62), (d) labile inorganic P (R^2=0.03; p=0.177; n=63) and (e) microbial biomass P (R^2=0.09; p=0.022; n=58). Outliers are indicated as crosses and were not included in regression analyses.

Supplementary Table S1. Results of correlation analysis for aboveground biomass, soil physicochemical properties, soil texture, soil total, labile and microbial biomass C, N and P pools, soil enzyme activities and P fluxes measured in pastures, secondary forests and old-growth forests (OGF) in the Agua Salud watershed, Panama. Values in boxes represent Pearson's r. Symbols above the values indicate significant correlations between the respective parameters (•, p<0.1, *, p<0.05, **, p<0.01, ***, p<0.001, n.s.=p>0.05).

Parameters	Soil pH	Oxalate Fe	Total dissolved N	Resin P	Microbial biomass C	Microbial biomass P	Phosphatase
Aboveground biomass	-0.45***	-0.52***	0.16^{ns}	-0.32*	0.01^{ns}	0.43***	0.59***
Soil water content	-0.05^{ns}	0.14^{ns}	0.02^{ns}	-0.19^{ns}	0.41***	0.51***	0.34**
Soil pH	-	0.51***	-0.45***	0.24•	0.05^{ns}	-0.08^{ns}	-0.64***
Sand content	-0.25*	-0.52***	0.23•	-0.18^{ns}	-0.09^{ns}	-0.09^{ns}	0.25*
Silt content	0.17^{ns}	0.49***	-0.27*	0.25*	0.07^{ns}	0.07^{ns}	-0.27*
Clay content	0.18^{ns}	-0.06^{ns}	0.21^{ns}	-0.26*	0.04^{ns}	0^{ns}	0.13^{ns}
Oxalate Al	0.07^{ns}	0.09^{ns}	0.04^{ns}	0.08^{ns}	0.12^{ns}	-0.24•	-0.04^{ns}
Oxalate Fe	0.51***	-	-0.46***	0.47***	0.13^{ns}	0.07^{ns}	-0.48***
Oxalate P	0.17^{ns}	0.45***	-0.39**	0.68***	-0.08^{ns}	0.1^{ns}	-0.58***
Exchangeable Al	-0.88***	-0.4**	0.2^{ns}	-0.12^{ns}	-0.03^{ns}	0.22•	0.5***
Exchangeable Ca	0.88***	0.55***	-0.56***	0.23•	0.14^{ns}	0.24•	-0.49***
Exchangeable K	0.28*	0.51***	-0.31*	0.2^{ns}	0.11^{ns}	-0.01^{ns}	-0.37**
Cation exchange capacity	0.59***	0.59***	-0.73***	0.47***	0.07^{ns}	0.35**	-0.44***
Base saturation	0.91***	0.54***	-0.39**	0.24•	0.12^{ns}	-0.11^{ns}	-0.55***
Soil organic C	-0.19^{ns}	-0.07^{ns}	0.04^{ns}	-0.29*	0.34**	0.43***	0.35**
Total N	0.05^{ns}	0.1^{ns}	-0.03^{ns}	-0.23•	0.38**	0.43***	0.27*
Total P	0.25*	0.57***	-0.27*	0.55***	0.03^{ns}	0.07^{ns}	-0.5***
Total inorganic P	0^{ns}	0.49***	-0.2^{ns}	0.53***	-0.04^{ns}	0.1^{ns}	-0.36**
Total organic P	0.28*	0.56***	-0.27*	0.53***	0.04^{ns}	0.06^{ns}	-0.5***
Soil C:N	-0.49***	-0.34**	0.14^{ns}	-0.16^{ns}	0.02^{ns}	0.12^{ns}	0.22•
Soil C:P	-0.37**	-0.53***	0.29*	-0.6***	0.08^{ns}	0.14^{ns}	0.65***
Soil N:P	-0.24•	-0.46***	0.26*	-0.6***	0.09^{ns}	0.13^{ns}	0.63***
Dissolved organic C	-0.57***	-0.48***	0.96***	-0.47***	-0.08^{ns}	-0.22•	0.64***
Total dissolved N	-0.45***	-0.46***	-	-0.45***	-0.13^{ns}	-0.29*	0.58***
Ammonium	0.07^{ns}	-0.11^{ns}	0.2^{ns}	-0.24•	-0.12^{ns}	-0.11^{ns}	0.2^{ns}
Nitrate	-0.05^{ns}	-0.11^{ns}	0.11^{ns}	-0.2^{ns}	-0.18^{ns}	0.01^{ns}	0.11^{ns}
Dissolved inorganic N	0.04^{ns}	-0.12^{ns}	0.19^{ns}	-0.25*	-0.15^{ns}	-0.09^{ns}	0.19^{ns}

Dissolved organic N	-0.45***	-0.37**	0.85***	-0.29*	-0.04^{ns}	-0.22$^{•}$	0.45***
Resin P	0.24$^{•}$	0.47***	-0.45***	-	-0.18^{ns}	0.02^{ns}	-0.54***
Labile C:N	0.04^{ns}	0.16^{ns}	-0.69***	0.34**	-0.03^{ns}	0.26*	-0.26*
Labile C:P	-0.41***	-0.47***	0.75***	-0.76***	-0.06^{ns}	-0.09^{ns}	0.74***
Labile N:P	-0.37**	-0.46***	0.81***	-0.71***	-0.11^{ns}	-0.14^{ns}	0.72***
Microbial biomass C	0.05^{ns}	0.13^{ns}	-0.13^{ns}	-0.18^{ns}	-	0.3*	0.16^{ns}
Microbial biomass N	0.02^{ns}	0.05^{ns}	0.13^{ns}	-0.27*	0.84***	0.29*	0.33**
Microbial biomass P	-0.08^{ns}	0.07^{ns}	-0.29*	0.02^{ns}	0.3*	-	0.11^{ns}
Microbial C:N	0.1^{ns}	0.18^{ns}	-0.49***	0.19^{ns}	0.29*	-0.03^{ns}	-0.36**
Microbial C:P	0.13^{ns}	0.04^{ns}	0.13^{ns}	-0.11^{ns}	0.36**	-0.71***	-0.06^{ns}
Microbial N:P	0.11^{ns}	-0.01^{ns}	0.27*	-0.18^{ns}	0.31*	-0.69***	0.06^{ns}
P-Monoesterase act.	-0.64***	-0.48***	0.58***	-0.54***	0.16^{ns}	0.11^{ns}	-
P-Diesterase act.	-0.44***	-0.39**	0.54***	-0.59***	0.08^{ns}	0.05^{ns}	0.9***
NA glucosaminidase act.	0.24$^{•}$	0.28*	0.14^{ns}	-0.05^{ns}	0.2^{ns}	0.02^{ns}	0.17^{ns}
ß-glucosidase act.	0.26*	0.37**	-0.1^{ns}	0.15^{ns}	0.22$^{•}$	0.14^{ns}	-0.07^{ns}
Leu aminopeptidase act.	0.18^{ns}	0.1^{ns}	0.03^{ns}	0.15^{ns}	0.04^{ns}	-0.01^{ns}	-0.13^{ns}
Enzyme C:P	0.57***	0.65***	-0.4**	0.5***	-0.06^{ns}	-0.02^{ns}	-0.66***
Enzyme C:N	0.04^{ns}	0.24$^{•}$	-0.16^{ns}	0.07^{ns}	0.13^{ns}	0.09^{ns}	-0.07^{ns}
Enzyme N:P	0.49***	0.38**	-0.26*	0.42***	-0.14^{ns}	-0.1^{ns}	-0.64***
Gross Pi influx	0.25*	0.09^{ns}	-0.13^{ns}	-0.07^{ns}	0.14^{ns}	0.08^{ns}	-0.36**
Gross Pi efflux	0.28*	0.03^{ns}	-0.1^{ns}	-0.17^{ns}	0.12^{ns}	0.15^{ns}	-0.24$^{•}$
Net abiotic Pi immobilization	-0.24$^{•}$	0.26*	-0.05^{ns}	0.2^{ns}	0.07^{ns}	-0.17^{ns}	0.03^{ns}
Net biotic Pi immobilization	0.25*	-0.25*	0.04^{ns}	-0.16^{ns}	-0.04^{ns}	0.18^{ns}	-0.07^{ns}

7. Summary (English)

Human activities, such as logging and conversion of forest into agricultural fields, have led to an ongoing degradation of primary tropical forests, and today regenerating (or secondary) forests make up more than 50% of the global tropical forested area. Tropical secondary forests act as future carbon (C) sinks and are important for biodiversity conservation, but their regrowth potential can be constrained by soil nutrient availability. While nitrogen (N)-fixing tree species can trigger significant inputs of N from

the atmosphere in early succession, phosphorus (P) can be obtained solely from soils, but usually exists in poorly available forms in strongly weathered tropical soils. Therefore, increasing P limitation of tree growth with progressing succession has been suggested but rarely observed, and detailed knowledge of how soil P cycling changes during tropical secondary succession, especially with regards to interactions with Fe- and Al-oxides and soil microbes, is lacking. Metal oxides are major sorption sites for P in soils, but Fe-oxides may become solubilized and liberate P in response to redox fluctuations, a process affected by C availability and microbial activity. Soil microbes can assist plants by solubilizing P from pools unavailable for plants, but they also compete for and immobilize P in their biomass, which often increases during succession. In order to test how microbes mediate soil P cycling during forest regrowth and how P interacts with metal oxides at different stages of succession, we conducted a chronosequence study in the lowland moist tropics in Agua Salud, Panama, spanning 63 sites including pastures, secondary forests of various ages and old-growth forests. Along the chronosequence we measured soil physicochemical properties (soil pH, texture, Al-/Fe-oxides measured as oxalate extractable Al/Fe, cation exchange capacity, base saturation, exchangeable Al and Ca etc.) as well as total, dissolved and microbial biomass C and N, and related them to changes in total, available and microbial biomass P, soil P sorption, gross P mobilization and hydrolytic soil enzyme activities. Forest regrowth led to increases in soil organic C and soil water content. Soil pH decreased steadily during succession and triggered increases in exchangeable Al, while base saturation was generally high and decreased non-significantly. Phosphorus pools (soil total P and resin P) and oxalate extractable Fe were correlated to each other and decreased with succession, indicating that P associated with Fe-oxides is lost during succession. The decrease in soil P was accompanied by a redistribution of P into soil microbial biomass, so that the contribution of microbial biomass P to total P increased from 6 to 12%. Surprisingly, microbial biomass P increased independently of microbial biomass C and N, leading to decreases in microbial C:P and microbial N:P ratios. At the same time, the activity of soil phosphatase enzymes increased with forest age, while enzymes involved in N and C cycling didn't change or even decreased slightly, respectively. The increase in phosphatase activity was related to decreases in various P pools (e.g. resin P) and increases in soil total and dissolved C:P ratios. On severely C and P impoverished soils

such as in this study microbial biomass growth may be primarily constrained by C and P availability. The re-establishment of litterfall in secondary forests can increase pulse events of high short-term C and P availability under which microbes store excess P in their biomass in order to be primed when labile C becomes available again. Our results suggest that P associated with Fe-oxides may contribute to short-term P availability due to increased Fe reduction potential in secondary forests where C inputs and soil moisture are elevated. Redistribution of P into microbial biomass can lead to aggravation of P limitation for plants with progressing succession, indicated by decreases in resin P and concomitant increases in phosphatase activity, with potential implications for the future C sink strength of late successional forests.

8. Zusammenfassung (Deutsch)

Die anhaltende Abholzung von tropischen Primärwäldern für menschliche Zwecke wie Holzgewinnung oder Umwandlung in landwirtschaftliche Flächen hat dazu geführt, dass heutzutage nachwachsende Sekundärwälder mehr als 50% der globalen Tropenwaldfläche ausmachen. Tropische Sekundärwälder fungieren als zukünftige Kohlenstoffspeicher und sind wichtig für den Erhalt der tropischen Biodiversität. Ihr Nachwuchspotenzial kann jedoch durch die Nährstoffverfügbarkeit des Bodens eingeschränkt werden. Während Stickstoff-fixierende Baumarten bedeutsame Mengen von Stickstoff (N) aus der Atmosphäre in Sekundärwälder eintragen können, kann Phosphor (P) ausschließlich aus Böden gewonnen werden, kommt in den stark verwitterten tropischen Böden jedoch in der Regel in schlecht verfügbarer Form vor. Daher wird vermutet, dass mit fortschreitender Sukzession Baumwachstum zunehmend durch geringe P-Verfügbarkeit eingeschränkt wird. Dies wurde jedoch selten direkt beobachtet und es fehlen detaillierte Kenntnisse darüber, wie sich der Boden-P-Zyklus während der tropischen Sekundärsukzession ändert, insbesondere im Hinblick auf Wechselwirkungen mit Fe- und Al-Oxiden und Bodenmikroben. Metalloxide sind wichtige Sorptionsstellen für P in Böden, Fe-Oxide können jedoch in Reaktion auf Reduktionsprozesse in Lösung gehen und P freisetzen. Dieser Prozess wird durch mikrobielle Aktivität und die Verfügbarkeit von Kohlenstoff (C) verstärkt.

Bodenmikroben können Pflanzen unterstützen, indem sie P aus für Pflanzen nicht verfügbaren Pools verfügbar machen. Sie konkurrieren jedoch auch um P und binden es in ihrer Biomasse, die häufig während der Sekundärsukzession zunimmt. Um zu testen, wie Bodenmikroben den P-Zyklus während des Nachwachsens von tropischen Wäldern beeinflussen und wie P in verschiedenen Stadien der Sukzession mit Metalloxiden interagiert, haben wir eine Chronosequenzstudie in den feuchten Tieflandtropen in Agua Salud, Panama, durchgeführt, die 63 Standorte umfasst, darunter Weiden, Sekundärwälder unterschiedlichen Alters und Primärwälder. Entlang der Chronosequenz wurden physikochemische Bodeneigenschaften (Boden-pH, Textur, Al-/Fe-Oxid-Gehalt, gemessen als Oxalat-extrahierbares Al/Fe, Kationenaustauschkapazität, Basensättigung, austauschbares Al und Ca usw.) sowie gesamter, gelöster und mikrobieller Biomasse C und N gemessen und in Relation mit Veränderungen des gesamten, verfügbaren und mikrobiellen Biomasse P, Boden-P-Sorption, Brutto-P-Mobilisierung und hydrolytischen Bodenenzymaktivitäten gesetzt. Das Nachwachsen der Wälder führte zu einer Erhöhung des Gehalts an organischem C im Boden und des Bodenwassergehalts. Der pH-Wert des Bodens nahm während der Sekundärsukzession stetig ab und führte zu einem Anstieg des austauschbaren Al^{3+} während die Basensättigung im Allgemeinen hoch war und nicht signifikant abnahm. Phosphorpools (Gesamter P im Boden und verfügbarer P) und Oxalat-extrahierbares Fe waren miteinander korreliert und nahmen im Zuge des Nachwachsens ab, was darauf hinweist, dass mit Fe-Oxiden assoziierter P während der Sekundärsukzession abnahm. Die Abnahme von P im Boden ging mit einer Umverteilung von P in mikrobielle Biomasse einher, so dass der Anteil von mikrobieller Biomasse P am gesamten P stetig zunahm. Überraschenderweise stieg der mikrobielle Biomasse P unabhängig vom mikrobiellen Biomasse C und N an, was zu einer Abnahme von mikrobiellen C:P und mikrobiellen N:P Verhältnissen führte. Gleichzeitig nahm die Aktivität von Phosphatase-Enzymen im Boden mit dem Alter des Waldes zu, während sich Aktivitäten von Enzymen, die am N- und C-Zyklus beteiligt sind, nicht veränderten bzw. sogar geringfügig verringerten. Die Zunahme der Phosphatase-Aktivität hing mit einer Abnahme verschiedener P-Pools (z. B. verfügbarer P) und einer Zunahme von gelösten C:P- und gesamten Boden C:P-Verhältnissen zusammen. Auf stark C und P verarmten Böden wie jene in dieser Studie ist das Wachstum von mikrobieller Biomasse hauptsächlich durch die Verfügbarkeit von C und P beschränkt. Mit der

Wiederherstellung von Streufall in Sekundärwäldern können sich Pulsereignisse vermehrt ereignen, unter denen sich die Verfügbarkeit von C und P kurzzeitig erhöht. Während solchen Pulsereignissen können Mikroben überschüssiges P in ihrer Biomasse speichern, um besser darauf reagieren zu können, wenn gelöster C wieder verfügbar wird. Unsere Ergebnisse legen nahe, dass mit Fe-Oxiden assoziierter P zu solchen kurzfristigen Erhöhungen in verfügbarem P beitragen kann. Dies könnte an einem erhöhten Fe-Reduktionspotenzial in Sekundärwäldern, in denen C-Einträge und Bodenfeuchtigkeit erhöht sind, liegen. Die Umverteilung von P in mikrobielle Biomasse kann bei Pflanzen mit fortschreitender Sukzession zu einer Verstärkung der Wachstumslimitierung durch P führen, was in dieser Studie durch eine Abnahme von verfügbarem P und einer gleichzeitigen Zunahme der Phosphatase-Aktivität angezeigt wird. Dies könnte Auswirkungen auf die künftige Kohlenstoffspeicherstärke von älteren Sekundärwäldern haben.

9. References

Acosta-Martínez, V., Cruz, L., Sotomayor-Ramírez, D., & Pérez-Alegría, L. (2007). Enzyme activities as affected by soil properties and land use in a tropical watershed. *Applied Soil Ecology*, *35*, 35–45. https://doi.org/10.1016/j.apsoil.2006.05.012

Adeleke, R., Nwangburuka, C., & Oboirien, B. (2017). Origins , roles and fate of organic acids in soils : A review. *South African Journal of Botany*, *108*, 393–406. https://doi.org/10.1016/j.sajb.2016.09.002

Albi, T., & Serrano, A. (2016). Inorganic polyphosphate in the microbial world. Emerging roles for a multifaceted biopolymer. *World Journal of Microbiology and Biotechnology*, *32*(27), 1–12. https://doi.org/10.1007/s11274-015-1983-2

Ancuceanu, R., Dinu, M., Hovaneţ, M. V., Anghel, A. I., Popescu, C. V., & Negreş, S. (2015). A survey of plant iron content-A semi-systematic review. *Nutrients*, *7*, 10320–10351. https://doi.org/10.3390/nu7125535

Angelo, E. D., Crutchfield, J., & Vandiviere, M. (2001). Rapid , sensitive , microscale determination of phosphate in water and soil. *Journal of Environmental Quality*, *30*, 2206–2209.

Barnard, R. L., Osborne, C. A., & Firestone, M. K. (2013). Responses of soil bacterial and fungal communities to extreme desiccation and rewetting. *The ISME Journal*, *7*(11), 2229–2241. https://doi.org/10.1038/ismej.2013.104

Batterman, S. A., Hedin, L. O., Van Breugel, M., Ransijn, J., Craven, D. J., & Hall, J. S. (2013). Key role of symbiotic dinitrogen fixation in tropical forest secondary succession. *Nature*, *502*,

224–227. https://doi.org/10.1038/nature12525

Bautista-Cruz, A., & del Castillo, R. F. (2005). Soil changes during secondary succession in a tropical montane cloud forest area. *Soil Sci. Soc. Am. J.*, *69*, 906–914. https://doi.org/10.2136/sssaj2004.0130

Berthrong, S. T., Jobbágy, E. G., & Jackson, R. B. (2009). A global meta-analysis of soil exchangeable cations, pH, carbon and nitrogen with afforestation. *Ecological Applications*, *19*(8), 2228–2241.

Bringhurst, K., & Jordan, P. (2015). The impact on nutrient cycles from tropical forest to pasture conversion in Costa Rica. *Sustainable Water Resources Management*, *1*, 3–13. https://doi.org/10.1007/s40899-015-0003-x

Brookes, P. C., Landman, A., Pruden, G., & Jenkinson, D. S. (1985). Chloroform fumigation and the release of soil nitrogen: A rapid direct extraction method to measure microbial biomass nitrogen in soil. *Soil Biology and Biochemistry*, *17*(6), 837–842. https://doi.org/10.1159/000477898

Brown, S., & Lugo, A. E. (1990). Tropical secondary forests. *Journal of Tropical Ecology*, *6*(1), 1–32. https://doi.org/10.1017/S0266467400003989

Cabugao, K. G., Timm, C. M., Carrell, A. A., Childs, J., Lu, T.-Y. S., Pelletier, D. A., … Norby, R. J. (2017). Root and rhizosphere bacterial phosphatase activity varies with tree species and soil phosphorus availability in Puerto Rico tropical forest. *Frontiers in Plant Science*, *8*, 1834. https://doi.org/10.3389/fpls.2017.01834

Camenzind, T., Hättenschwiler, S., Treseder, K. K., Lehmann, A., & Rillig, M. C. (2018). Nutrient limitation of soil microbial processes in tropical forests. *Ecological Monographs*, *88*(1), 4–21. https://doi.org/10.1002/ecm.1279

Campo, J., Solis, E., & Gallardo, J. (2012). Effects of fertilisation on soil nutrient characteristics and the growth of tree stand in secondary seasonally dry tropical forests in Mexico. *Journal of Tropical Forest Science*, *24*(3), 419–426.

Chacon, N., Silver, W. L., Dubinsky, E. A., & Cusack, D. F. (2006). Iron reduction and soil phosphorus solubilization in humid tropical forests soils: The roles of labile carbon pools and an electron shuttle compound. *Biogeochemistry*, *78*, 67–84. https://doi.org/10.1007/s10533-005-2343-3

Cleveland, C. C., & Liptzin, D. (2007). C:N:P stoichiometry in soil: is there a “Redfield ratio” for the microbial biomass? *Biogeochemistry*, *85*, 235–252. https://doi.org/10.1007/s10533-007-9132-0

Cleveland, C. C., Reed, S. C., & Townsend, A. R. (2006). Nutrient regulation of organic matter decomposition in a tropical rain forest. *Ecology*, *87*(2), 492–503. https://doi.org/10.1890/05-0525

Cleveland, C. C., & Townsend, A. R. (2006). Nutrient additions to a tropical rain forest drive substantial soil carbon dioxide losses to the atmosphere. *Proceedings of the National Academy of Sciences of the United States of America*, *103*(27), 10316–10321. https://doi.org/10.1073/pnas.0600989103

Cleveland, C. C., Townsend, A. R., & Schmidt, S. K. (2002). Phosphorus limitation of microbial processes in moist tropical forests: Evidence from short-term laboratory incubations and field studies. *Ecosystems*, *5*, 680–691. https://doi.org/10.1007/s10021-002-0202-9

Condit, R., Engelbrecht, B. M. J., Pino, D., Pérez, R., & Turner, B. L. (2013). Species distributions in response to individual soil nutrients and seasonal drought across a community of tropical trees. *Proceedings of the National Academy of Sciences*, *110*(13), 5064–5068. https://doi.org/10.1073/PNAS.1218042110

Cornell, R. M., Giovanoli, R., & Schneider, W. (1989). Review of the hydrolysis of iron(III) and the crystallization of amorphous iron(III) hydroxide hydrate. *Journal of Chemical Technology and Biotechnology*, *46*, 115–134.

Courchesne, F., & Turmel, M.-C. (2008). Extractable Al, Fe, Mn and Si. In M. Carter & E. Gregorich (Eds.), *Soil Sampling and Methods of Analysis* (2nd ed., pp. 307–315). Boca Raton, FL: Canadian Society of Soil Science, CRC Press. https://doi.org/10.2134/jeq2008.0018br

Cudennec, Y., & Lecerf, A. (2006). The transformation of ferrihydrite into goethite or hematite , revisited. *Journal of Solid State Chemistry*, *179*, 716–722. https://doi.org/10.1016/j.jssc.2005.11.030

Davidson, E.A., & Martinelli, L. A. (2009). Nutrient limitations to secondary forest regrowth. *Geophysical Monograph Series*, *186*, 299–309. https://doi.org/10.1029/2008GM000732

Davidson, Eric A., De Carvalho, C. J. R., Figueira, A. M., Ishida, F. Y., Ometto, J. P. H. B., Nardoto, G. B., … Martinelli, L. A. (2007). Recuperation of nitrogen cycling in Amazonian forests following agricultural abandonment. *Nature*, *447*, 995–998. https://doi.org/10.1038/nature05900

Davidson, Eric A, Carvalho, C. J. R. De, Vieira, I. C. G., Figueiredo, R. De, Moutinho, P., Ishida, F. Y., … Sabá, R. T. (2004). Nitrogen and phosphorus limitation of biomass growth in a tropical secondary forest. *Ecological Applications*, *14*(4), 150–163. https://doi.org/10.1890/01-6006

Deltombe, E., & Pourbaix, M. (1958). The electrochemical behaviour of aluminum - Potential pH diagram of the system Al-H2O at 25 C. *Corrosion*, *14*(11), 16–20.

Deng, Q., McMahon, D. E., Xiang, Y., Yu, C.-L., Jackson, R. B., & Hui, D. (2017). A global meta-analysis of soil phosphorus dynamics after afforestation. *New Phytologist*, *213*, 181–192. https://doi.org/10.1111/nph.14119

Dirzo, R., & Raven, P. H. (2003). Global state of biodiversity and loss. *Annual Review of Environment and Resources*, *28*, 137–167. https://doi.org/10.1146/annurev.energy.28.050302.105532

Duff, S. M. G., Sarath, G., & Plaxton, W. C. (1994). The role of acid phosphatases in plant phosphorus metabolism. *Physiologia Plantarum*, *90*, 791–800. https://doi.org/10.1111/j.1399-3054.1994.tb02539.x

Eaton, J. M., & Lawrence, D. (2009). Loss of carbon sequestration potential after several decades of shifting cultivation in the Southern Yucatán. *Forest Ecology and Management*, *258*, 949–958. https://doi.org/10.1016/j.foreco.2008.10.019

Esberg, C., du Toit, B., Olsson, R., Ilstedt, U., & Giesler, R. (2010). Microbial responses to P addition in six South African forest soils. *Plant and Soil*, *329*, 209–225. https://doi.org/10.1007/s11104-009-0146-3

Fanin, N., Hättenschwiler, S., Schimann, H., & Fromin, N. (2015). Interactive effects of C, N and P fertilization on soil microbial community structure and function in an Amazonian rain forest. *Functional Ecology*, *29*, 140–150. https://doi.org/10.1111/1365-2435.12329

FAO. (2010). Global Forest Resources Assessment 2010. *FAO Forestry Paper*, *163*, 350 pp.

https://doi.org/ISBN 978-92-5-106654-6

Feng, J., & Zhu, B. (2019). A global meta-analysis of soil respiration and its components in response to phosphorus addition. *Soil Biology and Biochemistry*, *135*, 38–47. https://doi.org/10.1016/j.soilbio.2019.04.008

Figueiredo, V., Enrich-Prast, A., & Rütting, T. (2019). Evolution of nitrogen cycling in regrowing Amazonian rainforest. *Scientific Reports*, *9*, 8538. https://doi.org/10.1038/s41598-019-43963-4

Fimmen, R. L., Richter, D. deB., Vasudevan, D., Williams, M. A., & West, L. T. (2008). Rhizogenic Fe–C redox cycling: A hypothetical biogeochemical mechanism that drives crustal weathering in upland soils. *Biogeochemistry*, *87*, 127–141. https://doi.org/10.1007/s10533-007-9172-5

Fleischer, K., Rammig, A., De Kauwe, M. G., Walker, A. P., Domingues, T. F., Fuchslueger, L., … Lapola, D. M. (2019). Amazon forest response to CO2 fertilization dependent on plant phosphorus acquisition. *Nature Geoscience*, *12*, 736–741. https://doi.org/10.1038/s41561-019-0404-9

Fujii, K. (2014). Soil acidification and adaptations of plants and microorganisms in Bornean tropical forests. *Ecological Research*, *29*, 371–381. https://doi.org/10.1007/s11284-014-1144-3

Gehring, C., Denich, M., Kanashiro, M., & Vlek, P. L. G. (1999). Response of secondary vegetation in Eastern Amazonia to relaxed nutrient availability constraints. *Biogeochemistry*, *45*, 223–241. https://doi.org/10.1007/BF00993001

Gehring, C., Vlek, P. L. G., De Souza, L. A. G., & Denich, M. (2005). Biological nitrogen fixation in secondary regrowth and mature rainforest of central Amazonia. *Agriculture, Ecosystems and Environment*, *111*, 237–252. https://doi.org/10.1016/j.agee.2005.06.009

Gilroy, J. J., Woodcock, P., Edwards, F. A., Wheeler, C., Baptiste, B. L. G., Medina Uribe, C. A., … Edwards, D. P. (2014). Cheap carbon and biodiversity co-benefits from forest regeneration in a hotspot of endemism. *Nature Climate Change*, *4*, 503–507. https://doi.org/10.1038/nclimate2200

Goldberg, S., & Sposito, G. (1984). A chemical model of phosphate adsorption by soils: I. Reference oxide minerals. *Soil Science Society of America Journal*, *48*, 772–783. https://doi.org/10.2136/sssaj1985.03615995004900020050x

Hayashi, S. N., Guimarães, I. C. V., Reis, C. J. C., & Davidson, E. (2012). Linking nitrogen and phosphorus dynamics in litter production and decomposition during secondary forest succession in the eastern Amazon. *Boletim Do Museu Paraense Emílio Goeldi. Ciências Naturais*, *7*(3), 283–295.

He, Z., & Zhu, J. (1998). Microbial utilization and transformation of phosphate adsorbed by variable charge minerals. *Soil Biology and Biochemistry*, *30*(7), 917–923. https://doi.org/10.1016/S0038-0717(97)00188-0

Heineman, K. D., Turner, B. L., & Dalling, J. W. (2016). Variation in wood nutrients along a tropical soil fertility gradient. *New Phytologist*, *211*(2), 440–454. https://doi.org/10.1111/nph.13904

Hendershot, W. H., & Duquette, M. (1986). A simple barium chloride method for determining cation exchange capacity and exchangeable cations. *Soil Science Society of America Journal*, *50*(3), 605–608. https://doi.org/10.2136/sssaj1986.03615995005000030013x

Hosonuma, N., Herold, M., De Sy, V., De Fries, R. S., Brockhaus, M., Verchot, L., … Romijn, E. (2012). An assessment of deforestation and forest degradation drivers in developing countries. *Environmental Research Letters*, *7*, 044009. https://doi.org/10.1088/1748-9326/7/4/044009

Huang, W. J., Zhou, G. Y., & Liu, J. X. (2012). Nitrogen and phosphorus status and their influence on aboveground production under increasing nitrogen deposition in three successional forests. *Acta Oecologica*, *44*, 20–27. https://doi.org/10.1016/j.actao.2011.06.005

Illmer, P., & Mutschlechner, W. (2004). Effect of temperature and pH on the toxicity of aluminium towards two new , soil born species of Arthrobacter sp. *Journal of Basic Microbiology*, *44*(2), 98–105. https://doi.org/10.1002/jobm.200310293

Jakovac, C. C., Peña-Claros, M., Kuyper, T. W., & Bongers, F. (2015). Loss of secondary-forest resilience by land-use intensification in the Amazon. *Journal of Ecology*, *103*, 67–77. https://doi.org/10.1111/1365-2745.12298

Johnson, S. E., & Loeppert, R. H. (2006). Role of organic acids in phosphate mobilization from iron oxide. *Soil Science Society of America Journal, 70*, 222–234. https://doi.org/10.2136/sssaj2005.0012

Johnston, E. R., Kim, M., Hatt, J. K., Phillips, J. R., Yao, Q., Song, Y., … Konstantinidis, K. T. (2019). Phosphate addition increases tropical forest soil respiration primarily by deconstraining microbial population growth. *Soil Biology and Biochemistry*, *130*, 43–54. https://doi.org/10.1016/J.SOILBIO.2018.11.026

Jones, D. L., & Willett, V. B. (2006). Experimental evaluation of methods to quantify dissolved organic nitrogen (DON) and dissolved organic carbon (DOC) in soil. *Soil Biology and Biochemistry*, *38*(5), 991–999. https://doi.org/10.1016/j.soilbio.2005.08.012

Kauffman, J. B., Cummings, D. L., Ward, D. E., & Babbitt, R. (1995). Fire in the Brazilian Amazon: 1. Biomass, nutrient pools, and losses in slashed primary forests. *Oecologia*, *104*(4), 397–408. https://doi.org/10.1007/BF00341336

Khan, I., Fahad, S., Wu, L., Zhou, W., Xu, P., Sun, Z., … Hu, R. (2019). Labile organic matter intensifies phosphorous mobilization in paddy soils by microbial iron (III) reduction. *Geoderma*, *352*, 185–196. https://doi.org/10.1016/j.geoderma.2019.06.011

Kochian, L. V, Hoekenga, O. A., & Pineros, M. A. (2004). How do crop plants tolerate acid soils? Mechanisms of aluminum tolerance and phosphorus efficiency. *Annual Review of Plant Biology*, *55*, 459–493. https://doi.org/10.1146/annurev.arplant.55.031903.141655

Kouno, K., Tuchiya, Y., & Ando, T. (1995). Measurement of soil microbial biomass phosphorus by an anion exchange membrane method. *Soil Biology and Biochemistry*, *27*(10), 1353–1357.

Lauber, C. L., Strickland, M. S., Bradford, M. A., & Fierer, N. (2008). The influence of soil properties on the structure of bacterial and fungal communities across land-use types. *Soil Biology and Biochemistry*, *40*(9), 2407–2415. https://doi.org/10.1016/j.soilbio.2008.05.021

Lawrence, D., & Schlesinger, W. H. (2001). Changes in soil phosphorus during 200 years of shifting cultivation in Indonesia. *Ecology*, *82*(10), 2769–2780.

Lebrija-Trejos, E., Pérez-García, E. A., Meave, J. A., Poorter, L., & Bongers, F. (2011). Environmental changes during secondary succession in a tropical dry forest in Mexico. *Journal of Tropical Ecology*, *27*, 477–489. https://doi.org/10.1017/S0266467411000253

Lin, Y., Bhattacharyya, A., Campbell, A. N., Nico, P. S., Pett-Ridge, J., & Silver, W. L. (2018).

Phosphorus fractionation responds to dynamic redox conditions in a humid tropical forest soil. *Journal of Geophysical Research: Biogeosciences*, *123*, 3016–3027. https://doi.org/10.1029/2018JG004420

Liptzin, D., & Silver, W. L. (2009). Effects of carbon additions on iron reduction and phosphorus availability in a humid tropical forest soil. *Soil Biology and Biochemistry*, *41*(8), 1696–1702. https://doi.org/10.1016/j.soilbio.2009.05.013

Liptzin, D., Silver, W. L., & Detto, M. (2011). Temporal dynamics in soil oxygen and greenhouse gases in two humid tropical forests. *Ecosystems*, *14*, 171–182. https://doi.org/10.1007/s10021-010-9402-x

Liu, L., Gundersen, P., Zhang, T., & Mo, J. (2012). Effects of phosphorus addition on soil microbial biomass and community composition in three forest types in tropical China. *Soil Biology and Biochemistry*, *44*, 31–38. https://doi.org/10.1016/j.soilbio.2011.08.017

Lodge, D. J., McDowell, W. H., & McSwiney, C. P. (1994). The importance of nutrient pulses in tropical forests. *Trends in Ecology and Evolution*, *9*(10), 384–387. https://doi.org/10.1016/0169-5347(94)90060-4

Lohbeck, M., Poorter, L., Martinez-Ramos, M., & Bongers, F. (2015). Biomass is the main driver of changes in ecosystem process rates during tropical forest succession. *Ecology*, *96*(5), 1242–1252. https://doi.org/10.1890/14-0472.1

Ma, X., Mason-Jones, K., Liu, Y., Blagodatskaya, E., Kuzyakov, Y., Guber, A., … Razavi, B. S. (2019). Coupling zymography with pH mapping reveals a shift in lupine phosphorus acquisition strategy driven by cluster roots. *Soil Biology and Biochemistry*, *135*(May), 420–428. https://doi.org/10.1016/j.soilbio.2019.06.001

Mackenzie, K. M., Robertson, D. R., Adams, J. N., Altieri, A. H., & Turner, B. L. (2019). Structure and nutrient transfer in a tropical pelagic upwelling food web : From isoscapes to the whole ecosystem. *Progress in Oceanography*, *178*, 102145. https://doi.org/10.1016/j.pocean.2019.102145

Maranguit, D., Guillaume, T., & Kuzyakov, Y. (2017). Effects of flooding on phosphorus and iron mobilization in highly weathered soils under different land-use types: Short-term effects and mechanisms. *Catena*, *158*, 161–170. https://doi.org/10.1016/j.catena.2017.06.023

Marklein, A. R., & Houlton, B. Z. (2012). Nitrogen inputs accelerate phosphorus cycling rates across a wide variety of terrestrial ecosystems. *New Phytologist*, *193*, 696–704. https://doi.org/10.1111/j.1469-8137.2011.03967.x

Marx, M.-C., Wood, M., & Jarvis, S. C. (2001). A microplate fluorimetric assay for the study of enzyme diversity in soils. *Soil Biology and Biochemistry*, *33*, 1633–1640.

Melton, E. D., Swanner, E. D., Behrens, S., Schmidt, C., & Kappler, A. (2014). The interplay of microbially mediated and abiotic reactions in the biogeochemical Fe cycle. *Nature Reviews Microbiology*, *12*, 797–808. https://doi.org/10.1038/nrmicro3347

Miller, A. J., Schuur, E. A. G., & Chadwick, O. A. (2001). Redox control of phosphorus pools in Hawaiian montane forest soils. *Geoderma*, *102*, 219–237. https://doi.org/10.1016/S0016-7061(01)00016-7

Miller, W. P., & Miller, D. M. (1987). A micro-pipette method for soil mechanical analysis. *Communications in Soil Science & Plant Analysis*, *18*, 1–15.

Nagy, R. C., Rastetter, E. B., Neill, C., & Porder, S. (2017). Nutrient limitation in tropical secondary

forests following different management practices. *Ecological Applications*, *27*(3), 734–755. https://doi.org/10.1002/eap.1478

Nakas, J. P., Gould, W. D., & Klein, D. A. (1987). Origin and expression of phosphatase activity in a semi-arid grassland soil. *Soil Biology and Biochemistry*, *19*, 13–18. https://doi.org/10.1016/0038-0717(87)90118-0

Nannipieri, P., Giagnoni, L., Landi, L., & Renella, G. (2011). Role of phosphatase enzymes in soil. In E. K. Bünemann, A. Oberson, & E. Frossard (Eds.), *Phosphorus in Action - Biological Processes in Soil Phosphorus Cycling* (Vol. 26, pp. 215–243). Heidelberg: Springer. https://doi.org/10.1007/978-3-642-15271-9

Neumann-Cosel, L., Zimmermann, B., Hall, J. S., van Breugel, M., & Elsenbeer, H. (2011). Soil carbon dynamics under young tropical secondary forests on former pastures-A case study from Panama. *Forest Ecology and Management*, *261*, 1625–1633. https://doi.org/10.1016/j.foreco.2010.07.023

Nottingham, A. T., Turner, B. L., Whitaker, J., Ostle, N. J., McNamara, N. P., Bardgett, R. D., … Meir, P. (2015). Soil microbial nutrient constraints along a tropical forest elevation gradient: A belowground test of a biogeochemical paradigm. *Biogeosciences*, *12*, 6071–6083. https://doi.org/10.5194/bg-12-6071-2015

Nottingham, Andrew T., Turner, B. L., Chamberlain, P. M., Stott, A. W., & Tanner, E. V. J. (2012). Priming and microbial nutrient limitation in lowland tropical forest soils of contrasting fertility. *Biogeochemistry*, *111*, 219–237. https://doi.org/10.1007/s10533-011-9637-4

Ogden, F. L., Crouch, T. D., Stallard, R. F., & Hall, J. S. (2013). Effect of land cover and use on dry season river runoff, runoff efficiency, and peak storm runoff in the seasonal tropics of Central Panama. *Water Resources Research*, *49*, 8443–8462. https://doi.org/10.1002/2013WR013956

Olander, L. P., & Vitousek, P. M. (2005). Short-term controls over inorganic phosphorus during soil and ecosystem development. *Soil Biology and Biochemistry*, *37*, 651–659. https://doi.org/10.1016/j.soilbio.2004.08.022

Olander, Lydia P., & Vitousek, P. M. (2000). Regulation of soil phosphatase and chitinase activity by N and P availability. *Biogeochemistry*, *49*, 175–19. https://doi.org/10.1023/A:1006316117817

Olander, Lydia P., & Vitousek, P. M. (2004). Biological and geochemical sinks for phosphorus in soil from a wet tropical forest. *Ecosystems*, *7*, 404–419. https://doi.org/10.1007/s10021-004-0264-y

Pan, Y., Birdsey, R., Fang, J., Houghton, R., Kauppi, P., Kurz, W., … Hayes, D. (2011). A large and persistent carbon sink in the world's forests. *Science*, *333*(6045), 988–993.

Parfitt, R. L. (1989). Phosphate reactions with natural allophane, ferrihydrite and goethite. *Journal of Soil Science*, *40*, 359–369. https://doi.org/10.1111/j.1365-2389.1989.tb01280.x

Parfitt, R. L., & Childs, C. W. (1988). Estimation of forms of Fe and Al : A review , and analysis of contrasting soils by dissolution and Moessbauer methods. *Australian Journal of Soil Research*, *26*, 121–144.

Peretyazhko, T., & Sposito, G. (2005). Iron(III) reduction and phosphorous solubilization in humid tropical forest soils. *Geochimica et Cosmochimica Acta*, *69*(14), 3643–3652. https://doi.org/10.1016/j.gca.2005.03.045

Poorter, L., Bongers, F., Aide, T. M., Almeyda Zambrano, A. M., Balvanera, P., Becknell, J. M., … Rozendaal, D. M. A. (2016). Biomass resilience of Neotropical secondary forests. *Nature*, *530*, 211–214. https://doi.org/10.1038/nature16512

Powers, J. S., & Marín-Spiotta, E. (2017). Ecosystem processes and biogeochemical cycles in secondary tropical forest succession. *Annual Review of Ecology, Evolution, and Systematics*, *48*, 497–519. https://doi.org/10.1146/annurev-ecolsys-110316-022944

Raich, J. W., Clark, D. A., Schwendenmann, L., & Wood, T. E. (2014). Aboveground tree growth varies with belowground carbon allocation in a tropical rainforest environment. *PLoS ONE*, *9*(6), e100275. https://doi.org/10.1371/journal.pone.0100275

Richardson, A. E., & Simpson, R. J. (2011). Soil microorganisms mediating phosphorus availability. *Plant Physiology*, *156*, 989–996. https://doi.org/10.1104/pp.111.175448

Richter, D. D., Allen, H. L., Li, J., Markewitz, D., & Raikes, J. (2006). Bioavailability of slowly cycling soil phosphorus: Major restructuring of soil P fractions over four decades in an aggrading forest. *Oecologia*, *150*, 259–271. https://doi.org/10.1007/s00442-006-0510-4

Robertson, G. P., & Tiedje, J. M. (1988). Deforestation alters denitrification in a lowland tropical rain forest. *Nature*, *336*(22), 756–759. https://doi.org/10.1038/336756a0

Rosinger, C., Rousk, J., & Sandén, H. (2019). Can enzymatic stoichiometry be used to determine growth-limiting nutrients for microorganisms ? - A critical assessment in two subtropical soils. *Soil Biology and Biochemistry*, *128*, 115–126. https://doi.org/10.1016/j.soilbio.2018.10.011

Sayer, E. J., Heard, M. S., Grant, H. K., Marthews, T. R., & Tanner, E. V. J. (2011). Soil carbon release enhanced by increased tropical forest litterfall. *Nature Climate Change*, *1*, 304–307. https://doi.org/10.1038/nclimate1190

Sayer, E. J., Joseph Wright, S., Tanner, E. V. J., Yavitt, J. B., Harms, K. E., Powers, J. S., … Turner, B. L. (2012). Variable responses of lowland tropical forest nutrient status to fertilization and litter manipulation. *Ecosystems*, *15*, 387–400. https://doi.org/10.1007/s10021-011-9516-9

Sayer, E. J., Powers, J. S., & Tanner, E. V. J. (2007). Increased litterfall in tropical forests boosts the transfer of soil CO2 to the atmosphere. *PLoS ONE*, *2*(12), e1299. https://doi.org/10.1371/journal.pone.0001299

Schimel, J., Becerra, C. A., & Blankinship, J. (2017). Estimating decay dynamics for enzyme activities in soils from different ecosystems. *Soil Biology and Biochemistry*, *114*, 5–11. https://doi.org/10.1016/j.soilbio.2017.06.023

Schreeg, L. A., Mack, M. C., & Turner, B. L. (2013). Leaf litter inputs decrease phosphate sorption in a strongly weathered tropical soil over two time scales. *Biogeochemistry*, *113*, 507–524. https://doi.org/10.1007/s10533-012-9781-5

Schwendenmann, L., & Veldkamp, E. (2005). The role of dissolved organic carbon, dissolved organic nitrogen, and dissolved inorganic nitrogen in a tropical wet forest ecosystem. *Ecosystems*, *8*, 339–351. https://doi.org/10.1007/s10021-003-0088-1

Schwertmann, U. (1991). Solubility and dissolution of iron oxides. *Plant and Soil*, *130*, 1–25. https://doi.org/10.1007/BF00011851

Serna-Chavez, H. M., Fierer, N., & van Bodegom, P. M. (2013). Global drivers and patterns of microbial abundance in soil. *Global Ecology and Biogeography*, *22*(10), 1162–1172. https://doi.org/10.1111/geb.12070

Seymour, F., & Harris, N. L. (2019). Reducing tropical deforestation. *Science*, *365*(6455), 756–757.

Siddique, I., Vieira, I. C. G., Schmidt, S., Lamb, D., Carvalho, C. J. R., Figueiredo, R. de O., … Davidson, E. A. (2010). Nitrogen and phosphorus additions negatively affect tree species diversity in tropical forest regrowth trajectories. *Ecology*, *91*(7), 2121–2131. https://doi.org/10.1890/09-0636.1

Sinsabaugh, R. L., Lauber, C. L., Weintraub, M. N., Ahmed, B., Allison, S. D., Crenshaw, C., … Zeglin, L. H. (2008). Stoichiometry of soil enzyme activity at global scale. *Ecology Letters*, *11*(11), 1252–1264. https://doi.org/10.1111/j.1461-0248.2008.01245.x

Smith, A. P., Marín-Spiotta, E., & Balser, T. (2015). Successional and seasonal variations in soil and litter microbial community structure and function during tropical postagricultural forest regeneration: a multiyear study. *Global Change Biology*, *21*, 3532–3547. https://doi.org/10.1111/gcb.12947

Sollins, P., Robertson, G. P., & Uehara, G. (1988). Nutrient mobility in variable- and permanent-charge soils. *Biogeochemistry*, *6*, 181–199.

Soong, J. L., Marañon-Jimenez, S., Cotrufo, M. F., Boeckx, P., Bodé, S., Guenet, B., … Janssens, I. A. (2018). Soil microbial CNP and respiration responses to organic matter and nutrient additions: Evidence from a tropical soil incubation. *Soil Biology and Biochemistry*, *122*, 141–149. https://doi.org/10.1016/J.SOILBIO.2018.04.011

Spohn, M., & Kuzyakov, Y. (2013). Phosphorus mineralization can be driven by microbial need for carbon. *Soil Biology and Biochemistry*, *61*, 69–75. https://doi.org/10.1016/J.SOILBIO.2013.02.013

Stefánsson, A. (2007). Iron(III) hydrolysis and solubility at 25°C. *Environmental Science and Technology*, *41*, 6117–6123. https://doi.org/10.1021/es070174h

Sullivan, B. W., Nifong, R. L., Nasto, M. K., Alvarez-Clare, S., Dencker, C. M., Soper, F. M., … Cleveland, C. C. (2019). Biogeochemical recuperation of lowland tropical forest during succession. *Ecology*, *100*(4), e02641. https://doi.org/10.1002/ecy.2641

Tang, C., & Rengel, Z. (2003). Role of plant cation/anion uptake ratio in soil acidification. In Z. Rengel (Ed.), *Handbook of Soil Acidity* (pp. 57–81). New York, NY; Basel, Switzerland: Marcel Dekker, Inc.

Tarafdar, J. C., & Claassen, N. (1988). Organic phosphorus compounds as a phosphorus source for higher plants through the activity of phosphatases produced by plant roots and microorganisms. *Biology and Fertility of Soils*, *5*, 308–312. https://doi.org/10.1007/BF00262137

Thompson, A., Chadwick, O. A., Rancourt, D. G., & Chorover, J. (2006). Iron-oxide crystallinity increases during soil redox oscillations. *Geochimica et Cosmochimica Acta*, *70*(7), 1710–1727. https://doi.org/10.1016/j.gca.2005.12.005

Tischer, A., Blagodatskaya, E., & Hamer, U. (2014). Extracellular enzyme activities in a tropical mountain rainforest region of southern Ecuador affected by low soil P status and land-use change. *Applied Soil Ecology*, *74*, 1–11. https://doi.org/10.1016/J.APSOIL.2013.09.007

Turner, B. L. (2010). Variation in ph optima of hydrolytic enzyme activities in tropical rain forest soils. *Applied and Environmental Microbiology*, *76*(19), 6485–6493. https://doi.org/10.1128/AEM.00560-10

Turner, B. L., Brenes-Arguedas, T., & Condit, R. (2018). Pervasive phosphorus limitation of tree

species but not communities in tropical forests. *Nature*, *555*, 367–370. https://doi.org/10.1038/nature25789

Turner, B. L., & Engelbrecht, B. M. J. (2011). Soil organic phosphorus in lowland tropical rain forests. *Biogeochemistry*, *103*, 297–315. https://doi.org/10.1007/s10533-010-9466-x

Turner, B. L., & Joseph Wright, S. (2014). The response of microbial biomass and hydrolytic enzymes to a decade of nitrogen, phosphorus, and potassium addition in a lowland tropical rain forest. *Biogeochemistry*, *117*(1), 115–130. https://doi.org/10.1007/s10533-013-9848-y

Turner, B. L., Lambers, H., Condron, L. M., Cramer, M. D., Leake, J. R., Richardson, A. E., & Smith, S. E. (2013). Soil microbial biomass and the fate of phosphorus during long-term ecosystem development. *Plant and Soil*, *367*, 225–234. https://doi.org/10.1007/s11104-012-1493-z

Turner, B. L., & Romero, T. E. (2010). Stability of hydrolytic enzyme activity and microbial phosphorus during storage of tropical rain forest soils. *Soil Biology and Biochemistry*, *42*(3), 459–465. https://doi.org/10.1016/j.soilbio.2009.11.029

Ushio, M., Fujiki, Y., Hidaka, A., & Kitayama, K. (2015). Linkage of root physiology and morphology as an adaptation to soil phosphorus impoverishment in tropical montane forests. *Functional Ecology*, *29*, 1235–1245. https://doi.org/10.1111/1365-2435.12424

van Breugel, M., Craven, D., Lai, H. R., Baillon, M., Turner, B. L., & Hall, J. S. (2019). Soil nutrients and dispersal limitation shape compositional variation in secondary tropical forests across multiple scales. *Journal of Ecology*, *107*, 566–581. https://doi.org/10.1111/1365-2745.13126

van Breugel, M., Hall, J. S., Craven, D., Bailon, M., Hernandez, A., Abbene, M., & Van Breugel, P. (2013). Succession of ephemeral secondary forests and their limited role for the conservation of floristic diversity in a human-modified tropical landscape. *PLoS ONE*, *8*(12). https://doi.org/10.1371/journal.pone.0082433

Vance, E. D., Brookes, P. C., & Jenkinson, D. S. (1987). An extraction method for measuring soil microbial biomass C. *Soil Biology and Biochemistry*, *19*(6), 703–707. https://doi.org/10.1016/0038-0717(87)90052-6

Vitousek, P. M., Porder, S., Houlton, B. Z., & Chadwick, O. A. (2010). Terrestrial phosphorus limitation: mechanisms, implications, and nitrogen–phosphorus interactions. *Ecological Applications*, *20*(1), 5–15. https://doi.org/10.1890/08-0127.1

Vogelsang, V., Fiedler, S., Jahn, R., & Kaiser, K. (2016). In-situ transformation of iron-bearing minerals in marshland-derived paddy subsoil. *European Journal of Soil Science*, *67*, 676–685. https://doi.org/10.1111/ejss.12374

Walker, T. W., & Syers, J. K. (1976). The fate of phosphorus during pedogenesis. *Geoderma*, *15*, 1–19. https://doi.org/10.1016/0016-7061(76)90066-5

Wanek, W., Zezula, D., Wasner, D., Mooshammer, M., & Prommer, J. (2019). A novel isotope pool dilution approach to quantify gross rates of key abiotic and biological processes in the soil phosphorus cycle. *Biogeosciences*, *16*, 3047–3068. https://doi.org/10.5194/bg-2018-519

Wang, Y.-P., Zhang, Q., Pitman, A. J., & Dai, Y. (2015). Nitrogen and phosphorous limitation reduces the effects of land use change on land carbon uptake or emission. *Environmental Research Letters*, *10*, 014001. https://doi.org/10.1088/1748-9326/10/1/014001

Waring, B. G., Weintraub, S. R., & Sinsabaugh, R. L. (2014). Ecoenzymatic stoichiometry of

microbial nutrient acquisition in tropical soils. *Biogeochemistry*, *117*, 101–113. https://doi.org/10.1007/s10533-013-9849-x

Weil, R. R., & Brady, N. C. (2017). *The Nature and Properties of Soils* (15th ed.). Essex, England: Pearson Education Limited.

Weiss, J. V., Emerson, D., & Megonigal, J. P. (2004). Geochemical control of microbial Fe(III) reduction potential in wetlands: Comparison of the rhizosphere to non-rhizosphere soil. *FEMS Microbiology Ecology*, *48*, 89–100. https://doi.org/10.1016/j.femsec.2003.12.014

Windsor, D. M. (1990). Climate and moisture variability in a tropical forest : Long-term records from Barro Colorado Island , Panama. *Smithsonian Contributions to the Earth Sciences*, *29*, 1–145.

Wolf, S., Eugster, W., Potvin, C., Turner, B. L., & Buchmann, N. (2011). Carbon sequestration potential of tropical pasture compared with afforestation in Panama. *Global Change Biology*, *17*, 2763–2780. https://doi.org/10.1111/j.1365-2486.2011.02460.x

Wright, S. J., Turner, B. L., Yavitt, J. B., Harms, K. E., Kaspari, M., Tanner, E. V. J., … Garcia, M. N. (2018). Plant responses to fertilization experiments in lowland, species-rich, tropical forests. *Ecology*, *99*(5), 1129–1138. https://doi.org/10.1002/ecy.2193

Xu, X., Thornton, P. E., & Post, W. M. (2013). A global analysis of soil microbial biomass carbon, nitrogen and phosphorus in terrestrial ecosystems. *Global Ecology and Biogeography*, *22*, 737–749. https://doi.org/10.1111/geb.12029

Yang, X., & Post, W. M. (2011). Phosphorus transformations as a function of pedogenesis: A synthesis of soil phosphorus data using Hedley fractionation method. *Biogeosciences*, *8*, 2907–2916. https://doi.org/10.5194/bg-8-2907-2011

Yao, Q., Li, Z., Song, Y., Wright, S. J., Guo, X., Tringe, S. G., … Pan, C. (2018). Community proteogenomics reveals the systemic impact of phosphorus availability on microbial functions in tropical soil. *Nature Ecology & Evolution*, *2*, 499–509. https://doi.org/10.1038/s41559-017-0463-5

Yokoyama, D., Imai, N., & Kitayama, K. (2017). Effects of nitrogen and phosphorus fertilization on the activities of four different classes of fine-root and soil phosphatases in Bornean tropical rain forests. *Plant and Soil*, *416*, 463–476. https://doi.org/10.1007/s11104-017-3225-x

Zangaro, W., de Assis, R. L., Rostirola, L. V., de Souza, P. B., Gonçalves, M. C., Andrade, G., & Nogueira, M. A. (2008). Changes in arbuscular mycorrhizal associations and fine root traits in sites under different plant successional phases in southern Brazil. *Mycorrhiza*, *19*, 37–45. https://doi.org/10.1007/s00572-008-0202-5

Zanin, L., Tomasi, N., Cesco, S., Varanini, Z., & Pinton, R. (2019). Humic substances contribute to plant iron nutrition acting as chelators and biostimulants. *Frontiers in Plant Science*, *10*, 675. https://doi.org/10.3389/fpls.2019.00675

Zarin, D. J., Davidson, E. A., Brondizio, E., Vieira, I. C., Sá, T., Feldpausch, T., … Denich, M. (2005). Legacy of fire slows carbon accumulation in Amazonian forest regrowth. *Frontiers in Ecology and the Environment*, *3*(7), 365–369. https://doi.org/10.1890/1540-9295(2005)003[0365:LOFSCA]2.0.CO;2

Zheng, T., Liang, C., Xie, H., Zhao, J., Yan, E., Zhou, X., & Bao, X. (2019). Rhizosphere effects on soil microbial community structure and enzyme activity in a successional subtropical forest. *FEMS Microbiology Ecology*, *95*(5), 1–11. https://doi.org/10.1093/femsec/fiz043