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Effects of nutrient addition and soil drainage on germination of N-fixing and non-N-fixing tropical dry forest tree species

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Abstract

To develop generalised predictions regarding the effects of atmospheric nitrogen (N) and phosphorus (P) deposition on vegetation communities, it is necessary to account for the impacts of increased nutrient availability on the early life history stages of plants. Additionally, it is important to determine if these responses (a) differ between plant functional groups and (b) are modulated by soil drainage, which may affect the persistence of added nutrients. We experimentally assessed seed germination responses (germination proportion and germination energy, i.e. time to germination) of commonly occurring N-fixing and non-N-fixing tropical dry forest tree species found in India to simulated N and P deposition in well-drained soils, as well as soils with impeded drainage. When soils were not allowed to drain, germination proportion declined with nutrient addition, while germination energy remained unchanged. Stronger declines in germination proportion were observed for N-fixing species. In free-draining soils, nutrient addition did not affect germination proportion in either functional group. However, we detected a trend of delayed germination with nutrient addition, especially in N-fixers. Our results suggest that nutrient deposition can lead to potential shifts in functional dominance and tree community composition of tropical dry forests in the long term through its effects on early life stages of trees, although the mechanisms underlying the observed germination responses remain unclear. Further, such effects are likely to be spatially variable across the geographic range in which tropical dry forests occur depending on soil drainage properties.

Introduction

The increased emission and subsequent atmospheric deposition of nitrogen (N) and phosphorus (P) compounds as a direct consequence of human activities have resulted in the fertilisation of ecosystems across the globe (Falkowski et al. 2000; Galloway et al. 2004, 2008). Evidence from numerous experimental, observational and simulation studies have demonstrated that these increases in plant available nutrients have the potential to effect considerable changes in vegetation communities, from altered individual plant growth to changes in community composition and diversity, as well as ecosystem productivity and function (Aerts et al. 1992; Holland et al. 1997; Stevens et al. 2004; Waldrop et al. 2004; Wassen et al. 2005; Elser et al. 2007; Vitousek et al. 2010; La Pierre and Smith 2014). With the current increases in atmospheric N and P deposition, and the absence of strategies to mitigate imminent future acceleration in these rates, quantifying the potential responses of vegetation communities to increased N and P availability is critical for the effective assessment of future vegetation trajectories.

Much of our current knowledge of the effects of increased nutrient availability on vegetation comes from studies conducted in temperate ecosystems (Matson et al. 1999; Siddique et al. 2010). Considerable gaps exist in our understanding of how tropical ecosystems will respond to this global change driver (Bobbink et al. 2010; Lu et al. 2010). Additionally, while studies have evaluated the impacts of nutrient addition on community-level processes, especially for the juvenile and adult stages of plants (e.g. Aerts et al. 1990; Rainey et al. 1999; Bedison and McNeil 2009; Venterink and Güsewell 2010), most do not explicitly account for potential changes in the earliest life history stage, i.e. germination. Changes in seed germination with fertilisation have the potential to affect the number, composition and relative abundances of individuals upon which post-germination community-level processes can act. Therefore, assessing the effects of nutrient deposition on the early post-reproductive stages of plants, such as germination, is important in quantifying the mid- to long-term impacts of N and P deposition on ecosystems (Ackerly and Bazzaz 1995; Callahan et al. 2008). Although water availability (Khurana and Singh 2004; Renzhong and Qiong 2004; Ronnenberg et al. 2008), temperature (Ronnenberg et al. 2008; Milbau et al. 2009), soil properties (Roem et al. 2002) and the intensity, as well as quality of available light (Metcalfe 1996; Broncano et al. 1998; Holl et al. 2000; Ceccon et al. 2003; Luna and Moreno 2009), are all well recognised as important determinants of germination success, the role of nutrient availability remains poorly quantified. As a considerable fraction of a seed's mass is made up of stored carbohydrate and nutrient reserves (Fenner and Thompson 2005), it can be argued that the availability of nutrients in the germination media or environment may have only a minimal effect, if any on germination success (e.g. Broncano et al. 1998; Roem et al. 2002). However, studies have shown that N and P addition can influence seed germination. These effects can be positive (Benech-Arnold et al. 2000; Plassmann et al. 2008) or negative (Carter 1967; Pesch and Pieterse 1982; Radford et al. 1989; Kraaij and Ward 2006; Haden et al. 2011a, b) and further, can be dependent on plant species and functional type (Ceccon et al. 2003; Sweeney et al. 2008; Ochoa-Hueso and Manrique 2010; Basto et al. 2015).

Many studies use germination proportion as an indicator of germination success. However, germination proportion may not be the only indicator of potential alterations to plant communities as a result of nutrient addition. Germination energy, which is a metric describing the rate of germination, can also play an important role. For example, lower germination energy, which translates to delayed germination, could result in new individuals germinating into an environment where other, unaffected species have already established, thus limiting local resource availability for the new germinants. Additionally, in strongly seasonal ecosystems with a distinct growing season, delayed germination may translate to less time for growth (Vargas et al. 2015). Therefore, a combined assessment of changes in both germination proportion (Gp hereafter) and germination energy (Gt hereafter) can provide more ecologically relevant interpretations of the consequences of nutrient deposition than interpretations based on either parameter alone.

We assessed the effects of N and P fertilisation on germination proportion and energy in multiple tropical dry forest tree species commonly found in India. Tropical dry forests are of both regional and global importance (Murphy and Lugo 1986; Powers and Tiffin 2010; McShea and Davies 2011), and, in India, they represent the country's largest forest type in terms of land cover (MoEF 1999). They are of great value in terms of biodiversity as well as human utility of the landscape for a number of ecosystem services, and it is, therefore, crucial to assess the possible effects that nutrient deposition may have on this ecosystem. To our knowledge, there are currently no studies which assess germination responses of the tree community of tropical dry forests in India which can be generalised at the scale of the ecosystem.

This study assessed how seed germination responses to N and P deposition differed between two functional groups, i.e. N-fixers and non-N-fixers, both of which are prevalent in the tree communities of dry forests. Although N-fixing tropical dry forest species have been shown to have greater germination success and energy when compared to non-N-fixers (Vargas et al. 2015), the effects of enhanced nutrient deposition on the germination success of dry forest species belonging to these different functional groups remain unknown. Additionally, we sought to quantify if observed responses to nutrient addition were contingent on soil drainage properties.

Tropical dry forests have an extensive range in southern and central India, encompassing substantial topographic variation. Widespread and common tree species thus experience varying conditions of soil drainage during germination. By influencing rates of nutrient leaching (Howarth et al. 1996; Sims et al. 1998), soil drainage properties may modulate the duration of exposure and, therefore, the effects of added nutrients on seed germination. Assessing the variation in these effects can, therefore, provide insights into how tropical dry forest vegetation will respond to nutrient deposition across their geographic range.

Materials and methods

Species selection

Commonly occurring N-fixing and non-N-fixing tropical dry forest tree species from central and southern India were selected from published literature (e.g. Puyravaud et al. 1994; Sagar and Singh 2004; Kumar and Shahabuddin 2005; Kodandapani et al. 2008). Nodulation behaviour for all N-fixing species was confirmed using the Germplasm Resource Information Network database

(GRIN; http://www.ars-grin.gov/). Of the 19 species chosen for this study, the 10 N-fixing species belonged to a single family (Fabaceae), while the nine non-N-fixers represented eight families from seven orders. Seeds for each species were procured from a commercial supplier and were harvested no more than 6 months prior to the start of the experiments. Following procurement, all seeds were stored in cool, dry and dark conditions until the start of the experiments.

Experiment 1: Undrained germination medium

Seeds of eight tree species including four N-fixers (*Acacia ferruginea* DC., *Albizia amara* (Roxb.) B.Biovin, *Albizia lebbeck* (L.) Benth. and *Dalbergia latifolia* Roxb.) and four non-N-fixers (*Sapindus emarginatus* Vahl., *Shorea robusta* Gaertn., *Terminalia arjuna* (Roxb. ex DC.) Wight and Arn. and *Ziziphus jujuba* Mill.) were chosen for this study. The experiment was carried out in 15-cm-diameter food-grade plastic containers containing 150 g of sieved 1:1 sand and soil mixture.

The experiment consisted of five treatment combinations—a control (no nutrient addition) and a factorial combination of two levels of N (1 g m^{-2} N and 2 g m^{-2} N) and P (0.1 g m^{-2} P and 0.2 g m^{-2} P) addition. The two levels for each nutrient represent estimates of current and future deposition rates (expected by approximately 2030) as reported by Dentener et al. (2006) and Mahowald et al. (2008) for N and P, respectively. For convenience, we refer to these treatment combinations as follows: control (N0P0), low nitrogen–low phosphorus (NLPL), low nitrogen–high phosphorus (NLPH), high nitrogen–low phosphorus (NHPL) and high nitrogen–high phosphorus (NHPH). N and P were added to the germination media as a solution of urea and single superphosphate (SSP; $Ca(H_2PO_4)_2{\cdot}H_2O$), respectively, dissolved in distilled water. The amounts of nutrients to be added for different treatments were calculated based on the cross-sectional area of the germination containers (i.e. 0.018 m^2). The control treatment only received distilled water. Nutrient solutions were added to germination containers as a one-time application at the start of the experiment, simulating a pulse of wet deposition during the first rains of the wet season, which coincides with the time when species typically germinate. The total amount of liquid (water and/or nutrient solution) totalled 50 ml per germination container. Each germination container contained 10 seeds of a single species, with six replicates for each species–treatment combination (*N* = 240 germination containers in total). Each germination container was covered with a lid that allowed for ventilation through three 0.5-cm-diameter holes. The experiment was carried out within a laboratory setting (at the National Centre for Biological Sciences (NCBS), Bangalore, India), with temperature ranging between 20 and 30 °C and ambient light conditions with approximately 12-h day–night cycle.

The experiment ran for a period of 45 days, between May and June, 2012. Data were collected daily for the first 14 days, on alternate days for the next 22 days, and every third day for the remainder of the experiment. On data collection days, each germination container was scored for the total number of seeds germinated. We used radicle emergence as the criterion for germination success. Throughout the experiment, moisture content of the germination media was maintained at a saturated, but not inundated, level. At each sampling period, one germination container for each species was picked at random and tilted to observe if a film of water accumulated at the edge of the container. If the film of water did not form, 5 ml of distilled water was added to all germination containers for that species. The germination medium was not allowed to drain. Therefore, the loss of moisture could be attributed solely to evaporation via the ventilation holes on the lid of germination containers.

Experiment 2: Free-draining germination medium

This experiment used a set of nine N-fixers (*Acacia catechu* (L.f.) Willd., *Acacia ferruginea* DC., *Acacia nilotica* (L.) Delile, *Albizia amara* (Roxb.) B.Biovin., *Acrocarpus fraxinifolius* Arn., *Bauhinia purpurea* L., *Butea monosperma* (Lam.) Taub., *Dalbergia latifolia* Roxb. and *Pongamia* *pinnata* (L.) Pierre) and seven non-N-fixers (*Hardwickia binata* Roxb., *Lagerstroemia indica* L., *Lagerstroemia speciosa* (L.) Pers., *Phyllanthus emblica* L., *Terminalia arjuna* (Roxb. ex DC.) Wight and Arn.), *Wrightia tinctoria* R.Br. and *Ziziphus jujuba* Mill.).

The experiment was carried out in the same 15-cm-diameter food-grade plastic containers as were used for experiment 1. However, in this case, 300 g of a sieved 1:1 sand and soil mixture was used as the germination medium within each container. In contrast to experiment 1, the germination containers were not covered with a lid. Also, to allow the germination media to drain, outlet holes were drilled into the underside of each container.

Nutrient treatments consisted of factorial combinations of three levels of N (NO = 0 g m^{-2} N, NL = 2 g m^{-2} N and NH = 4 g m^{-2} N) and P (PO = 0 g m^{-2} P, PL = 0.2 g m^{-2} P and PH = 0.4 g m^{-2} P) addition as dissolved urea and SSP, respectively. On an area basis, NH and PH represent a doubling of N and P addition from those used in experiment 1. However, as experiment 2 used twice the volume of soil, the concentrations of N and P represented in these treatments are identical to those in experiment 1. Germination containers received a total of 50 ml of water and/or nutrient solutions at the start of the experiment. Similar to experiment 1, each germination container contained 10 seeds of a single species and each species–treatment combination was represented by six replicates, totalling 846 germination containers across the experiment. Running for a total of 50 days between October and December, 2013, data collection (i.e. number of seeds germinated in each germination container) was conducted daily for the first 12 days, on alternate days for the next 18 days and every third to fourth day for the remainder of the experiment. The experiment was carried out in a greenhouse at the NCBS campus. Temperature and relative humidity of the greenhouse were maintained at 28 °C and 60 %, respectively, for the duration of the experiment, with ambient light conditions and approximately 12-h day–night cycle. The greenhouse fogger system was used to supply germination containers with water.

To promote germination, seeds used in both experiments were soaked in distilled water at room temperature for a period of 36 h. prior to the start of the experiments. The pretreatment method and duration was informed by preliminary work carried out to identify a single pretreatment method sufficient to initiate germination across all species.

Data analysis

Gp was quantified as the proportion of seeds in each container that had germinated by the end of each experiment (45 and 50 days for experiments 1 and 2, respectively). For Gt, which represents germination energy, a single cumulative germination curve over time was constructed for each species–treatment combination by summing the number of seeds germinated in all six replicates for each experiment. We then estimated the time point (in days) at which 50 % (of total germination) of seeds in that species–treatment combination were observed to have germinated.

As a combined assessment of changes in germination proportion and energy can provide more ecologically meaningful interpretations of nutrient-mediated changes in germination, we propose a framework (Fig. 1) where Gp and Gt are interpreted simultaneously. Using log response ratios (LRRs) of Gp (LGp) and Gt (LGt), the response space can be divided into four quadrants centred around zero (representing no difference from the control treatment). Positive values of LGp with nutrient addition indicate an increase in germination proportion with respect to the control, while negative values indicate a decline. For LGt, negative values indicate earlier germination, while positive values suggest a delay. Following nutrient addition, a plant species or functional group that populates the upper left quadrant (quadrant II, where $LGp > 0$ and $LGt < 0$) stands to gain a distinct advantage ecologically, as it shows higher proportions and faster rates of germination. On the other hand, species or functional groups that populate the lower right of the response space (quadrant IV, where $LGp < 0$ and $LGt > 0$) may be at a disadvantage since they have a lower number of seeds germinating, which additionally take longer to germinate.

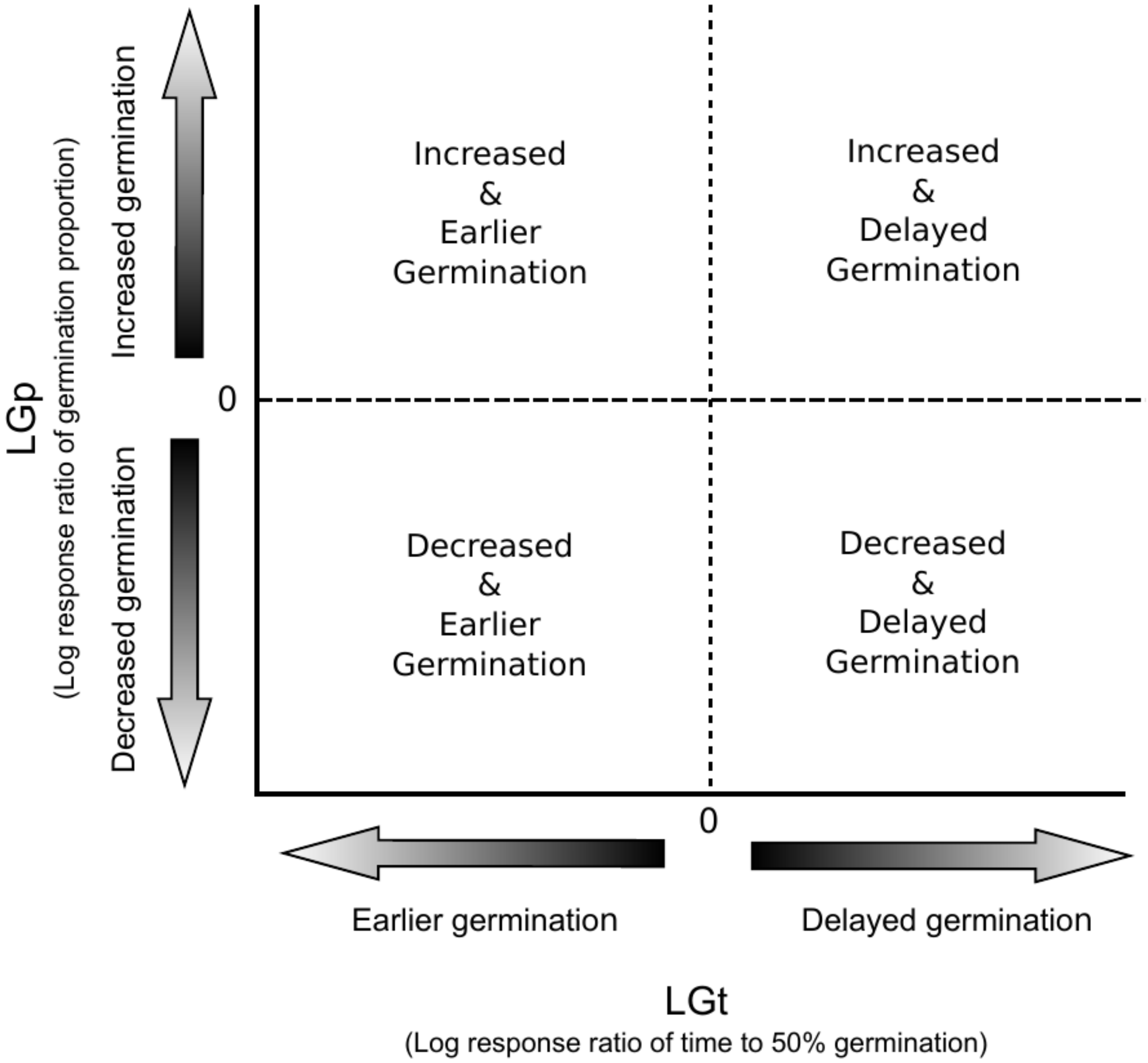

Fig. 1 Framework for the combined interpretation of changes in germination proportion and germination energy

As germination containers were not paired in either of the experiments, the LRR of Gp (LGp) for each functional group–treatment combination was calculated using a meta-analysis framework outlined by Hedges et al. (1999). This method accounts for and incorporates the variation between replicates, yielding mean LRRs and 95 % confidence intervals (CIs) which are weighted by within-species as well as between-species variability in responses. However, we note that when calculated for small sample sizes, the probability content of CIs could be as low as 91 % (Hedges et al. 1999). LRR of Gt (LGt) was calculated such that for a given species–treatment combination, LGt = $\ln(Gt_{\text{treatment}} / Gt_{\text{control}})$. LGt values for species were pooled by functional group to calculate the mean LGt for each treatment level as well as their associated 95 % CIs for N-fixers and non-N-fixers separately.

Using the framework outlined in Fig. 1, LGp and LGt values were plotted against each other (aggregated by plant functional group) to facilitate interpretation of germination responses using both these germination parameters simultaneously.

All statistical inferences in this study are based on the interpretation of calculated 95 % CIs around LRRs. LRRs of treatments where CIs do not overlap with zero are deemed to display strong differences in germination parameters from their respective controls. However, LRRs where 95 % CIs overlap with zero may also represent biologically meaningful changes in germination parameters and, hence, we do not assume a complete lack of nutrient-mediated effects. In scenarios where consistent (e.g. unidirectional) patterns of change in seed germination parameters are observed across nutrient treatments, but where 95 % CIs span zero, we report counternull values in addition to effect sizes as advocated by a number of authors (Rosenthal and Rubin 1994; Stephens et al. 2007; Rinella and James 2010). According to convention, we report counternull values which are two times the mean effect size. As an example, for a particular nutrient treatment, we may observe an LGp value of −0.6 (45 % decline in germination proportion relative to control) associated with 95 % CIs which range from 0.1 (10 % increase in germination) to −1.4 (75 %

decline in germination). The counternull value in this case is −1.2 (70 % decline in germination) and has the same amount of support as a response ratio value of 0, i.e. no difference from control. Therefore, the counternull value represents the non-null effect size which has as much statistical support as the null hypothesis of no change, and circumvents a common error in null hypothesis significance testing, where failure to reject the null hypothesis is equated with an effect size of zero (Rosenthal and Rubin 1994).

All analyses described here were carried out using R, version 3.0.2 (The R Foundation of Statistical Computing Platform 2013).

Results

Experiment 1: Undrained germination medium

N-fixers displayed strong declines in germination proportion across all nutrient treatments (i.e. negative LGp values with CIs that do not overlap zero) relative to controls (Fig. 2a; Supplementary Table S1). The strongest reductions were recorded for the NHPH treatment (−87.4 %), followed by the NHPL (−75.7 %), NLPH (−60 %) and NLPL (−50.5 %) treatments.

A reduction in germination proportion was also observed in non-N-fixers across all nutrient addition treatments, but these were not as strong as those observed in the N-fixers, with 95 % CIs spanning zero in all cases (Fig. 2b; Supplementary Table S1). However, effect sizes and associated counternull values suggest that the declines in germination proportion were large enough to be potentially biologically relevant. As with N-fixers, the strongest declines in germination proportion for non-N-fixers were recorded for the NHPH treatment (−68 %, counternull = −90 %), followed by the NHPL (−43 %, counternull = −68 %), NLPH (−35 %, counternull = −57 %) and NLPL (−34 %, counternull = −56 %).

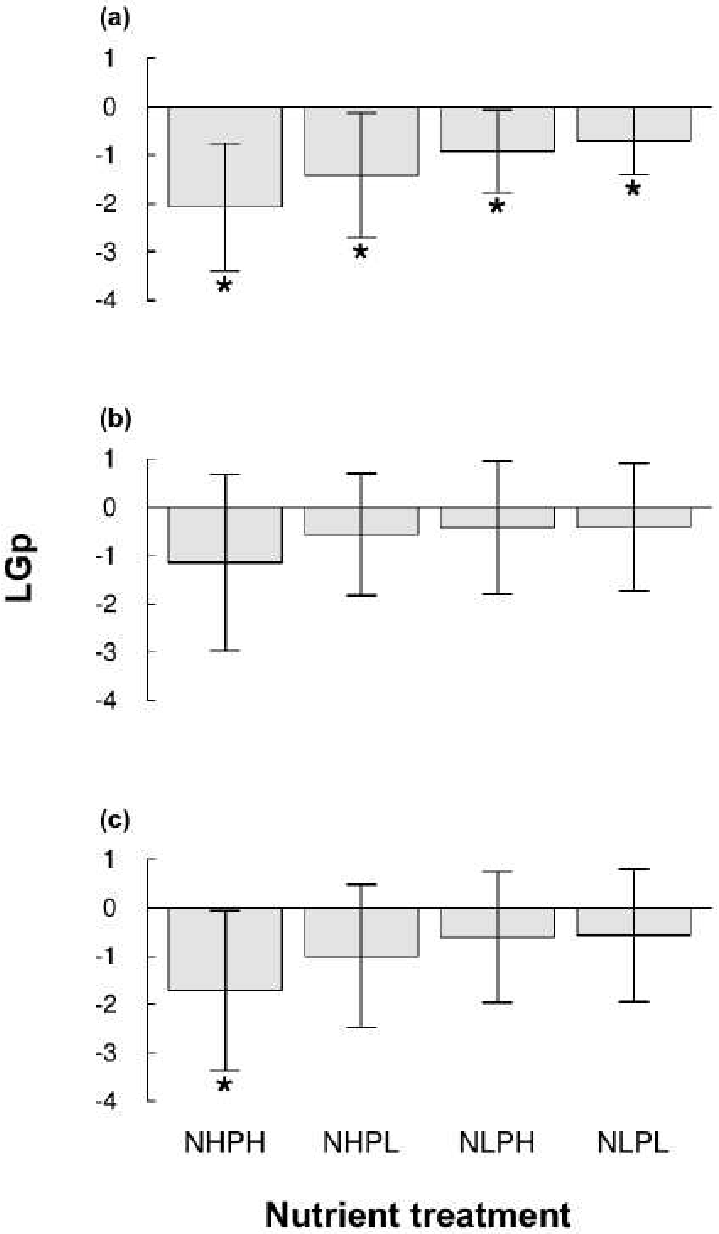

Fig. 2 Log response ratios representing changes in germination proportion (LGp) with nutrient addition in undrained soils for (**a)** N-fixers, (**b)** non-N-fixers and (**c)** all eight species combined. *Error bars* represent 95 % CIs around log response ratios. *Asterisks* (*) indicate treatment combinations where 95 % CIs do not overlap with 0

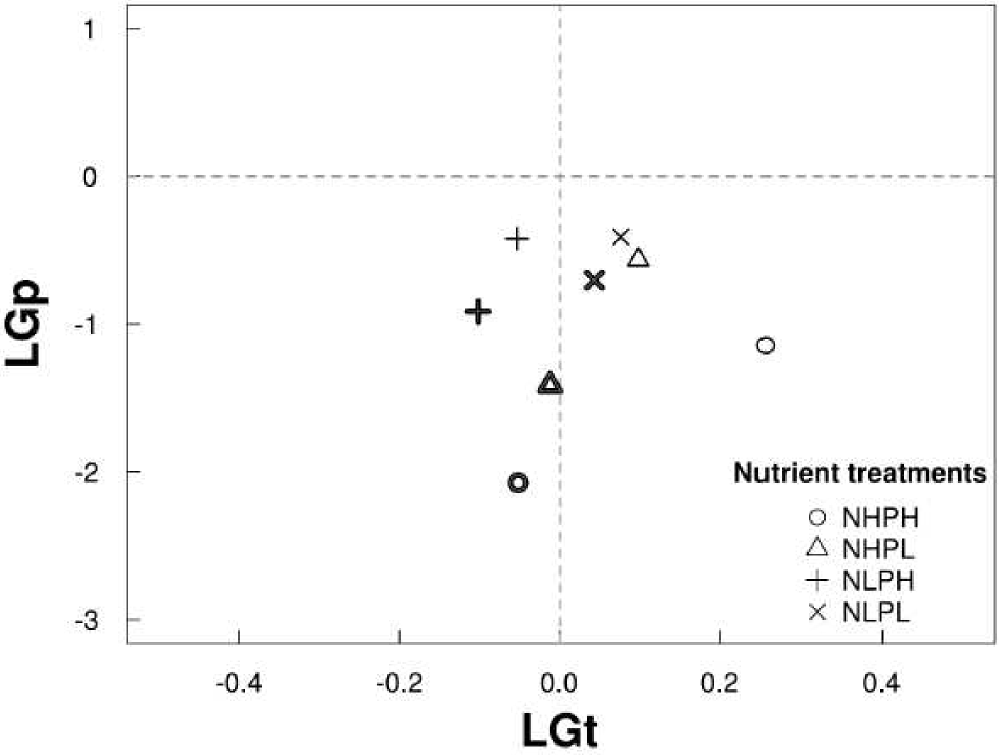

Fig. 3 Germination proportion (LGp) and germination energy (LGt) response space for undrained soils with nutrient addition. Non-N-fixers and N-fixers are represented by light and heavy plotting characters, respectively. For clarity, 95 % CIs around log response ratios are reported in supplementary Table S1

Similar patterns of reduced germination proportion were also observed when LGp values were calculated across all eight species used in the experiment (Fig. 2c; Supplementary Table S1). While the strongest declines in germination proportion were observed for the NHPH treatment (−82 %), CIs for the remaining three treatments spanned zero (NHPL = −63 %, counternull= −86 %; NLPH = −46 %, counternull = −71 %; NLPL = −44 %, counternull = −68 %).

There was no effect of nutrient addition on germination energy. Species were highly variable in their responses. Mean LGt values clustered around zero (Fig. 3) and CIs for all species combined, as well as for species pooled by functional groups, spanned zero (Supplementary Table S1).

Experiment 2: Free-draining germination medium

Nutrient addition treatments in free-draining soils did not elicit a response in either functional group with respect to germination proportion (Fig. 4; Supplementary Table S2). LGp values clustered close to zero, CIs were large and spanned zero in all cases and we failed to detect a discernible pattern in the direction of nutrient-mediated effects. At the species level, LGp values were associated with high variability, and although eight species–treatment combinations did show strong differences from control treatments, no consistent pattern was detected.

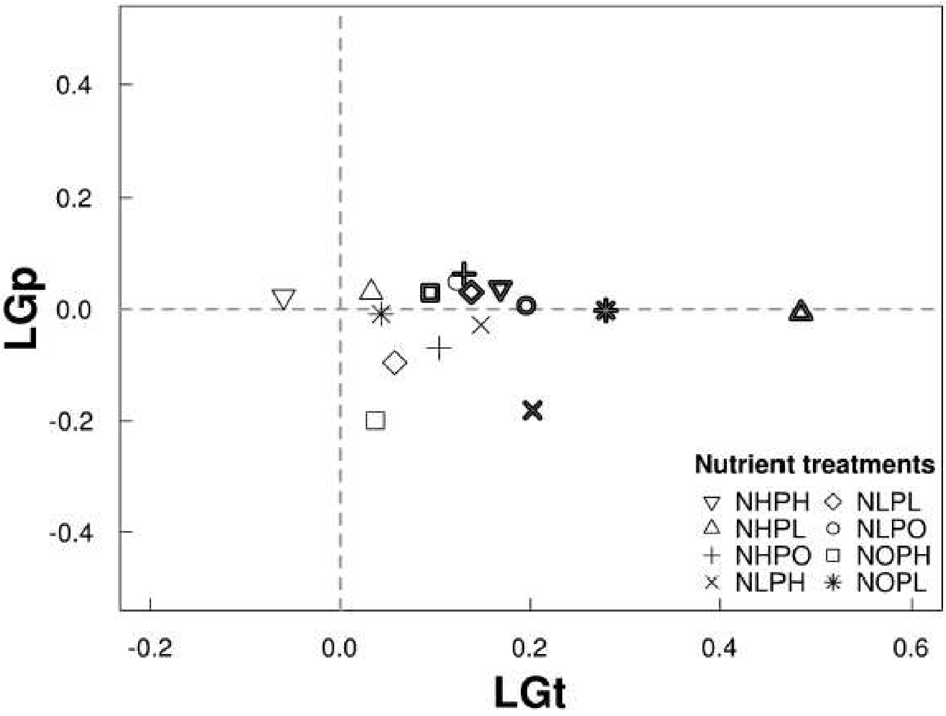

Fig. 4 Germination proportion (LGp) and germination energy (LGt) response space for free-draining soils with nutrient addition. Non-N-fixers and N-fixers are represented by light and heavy plotting characters, respectively. For clarity, 95 % CIs around log response ratios are reported in Supplementary Table S2

In terms of LGt, nutrient addition resulted in high variation in germination energy for both functional groups across all treatments. Ninety-five percent CIs overlapped zero in all cases, except the NLPO treatment in non-N-fixers (2 g m^{-2} N and no P addition) which showed a strong delay (13 %) in germination. Mean LGt values tended to cluster on the positive side of the LGt axis (Fig. 4; Supplementary Table S2), suggestive of an overall trend towards delayed germination. N-fixers displayed a stronger trend of delayed germination (24 %, counternull = 53 %) when averaged over all nutrient amendment treatments compared to non-N-fixers (6 %, counternull = 13 %). Across all species, germination was delayed by approximately 15 % (counternull = 34 %) when averaged over all nutrient addition treatments.

Discussion

From our experiments, we observe that in poorly draining soils, or when soils remain saturated for long periods of time, N and P addition to the germination medium resulted in a consistent decline in germination proportion for both functional groups, but had no effect on germination energy. While we detected stronger declines in germination proportion for N-fixing species, the magnitude of these declines in non-N-fixers, though weaker, may still be biologically meaningful. In well-drained soils, germination proportion was not affected by nutrient addition. However, we detected a trend of lower germination energy, with N-fixers showing greater delays in germination than non-N-fixers.

Our results suggest that in poorly draining soils, increased N and P deposition may result in community-level shifts in the recruiting tree community favouring non-N-fixing species. Further, these community-level changes may occur in conjunction with reduced seed germination in general, especially with high levels of N and P deposition. In well-drained soils, although we did not detect a change in germination proportion with nutrient addition, N and P addition could potentially result in community-level shifts in recruitment favouring non-N-fixers due to a stronger trend of delayed germination observed in N-fixers. We interpret these results from well-drained soils with caution, as responses were highly variable and associated with large confidence intervals. The detected trend of lower germination energy implies that new individuals may germinate into a community where potential competitors may have already germinated and would, therefore, be better equipped to exploit local resource availability. On account of the greater delays we observe in N-fixing species, individuals of this functional group may be more adversely affected than non-N-fixers, potentially resulting in shifts in functional diversity as post-germination community interactions are realised. However, we also note that our metric of change in germination energy is represented as a percentage. Therefore, species with an inherently fast germination time may not be adversely affected by the magnitude of germination delays we have quantified. For example, fast germinating species such as *D. latifolia* and *B. monosperma* may only see a germination delay of approximately 0.5 days, on average, with nutrient addition, while the relatively slower germinating *A. fraxinifolius* may show an average delay of 2.5 days (see Supplementary Tables S3, S4).

Previous studies from a diversity of ecosystems have reported a range of responses of seed germination proportion to nutrient availability in the germination medium. Some of these results have been consistent with our observations in poorly draining soils. In a study that assessed the role of N fertilisation in controlling bush encroachment in African savannas, Kraaij and Ward (2006) reported a strong reduction of germination proportion in *Acacia mellifera* seeds when exposed to nitrogen addition. In agricultural research, Haden et al. (2011a, b) demonstrated the negative influence of urea on germination of rice. Similarly, N additions in the form of urea, and to a lesser extent P additions as superphosphate, were reported to reduce germination in 10 crop and fodder species (Carter 1967). The role of N addition in reducing seed germination has also been proposed as a control measure for agricultural weeds (Pesch and Pieterse 1982; Dzomeku and Murdoch 2007) and parasitic plants (Irmaileh 1994). On the other hand, a number of studies have also reported no change in germination proportion with nutrient addition (Broncano et al. 1998; Roem et al. 2002), as well as stimulation, resulting in an increase in germination (Benech-Arnold et al. 2000; Plassmann et al. 2008).

Overall, the effects of nutrient availability on seed germination appear to be strongly contingent on species identity and functional group (Davis 2007; Ochoa-Hueso and Manrique 2010), and the mechanisms which bring about these differential changes in germination are currently not clear. Although the selected non-N-fixing species in these experiments were more phylogenetically diverse than the N-fixers, variability in responses to fertilisation was comparable between the two functional groups (Supplementary Tables S1, S2). The observed differences between N-fixers and non-N-fixers are thus unlikely to be a consequence of greater phylogenetic relatedness between N-fixers when compared to non-N-fixers.

Ochoa-Hueso and Manrique (2010) suggest that potential mechanisms of nutrient-mediated changes in germination could be categorised broadly into classes, such as signal effect, toxicity and microbial activity. Signal effects are instances when nutrient availability acts as a cue for germination. These can either be stimulatory, as proposed for both nitrate (Luna and Moreno 2009) and phosphate (Bell et al. 1993), whose availability increases in the soil after fires and may signal favourable germination conditions, or inhibitory cues, as observed for ammonium and phosphate in clover broomrape seeds (Yoneyama et al. 2001). By far, reductions in germination with nutrient addition have largely been attributed to toxicity, especially with respect to added N in the form of ammonia or urea (Bremner 1995), which itself dissociates to release ammonium. The level of nutrient supply can also have a strong bearing on germination, such that low to intermediate levels of N addition can stimulate germination, but high dosages can result in toxicity and reduced germination (Pérez-Fernández and Rodríguez-Escheverría 2003; Luna et al. 2007). Mechanisms invoking microbial activity involve a more indirect role of nutrients. Here, addition of nutrients to a germination medium that is severely nutrient limited stimulates the soil microbial community which then decompose seeds as they provide a rich source of carbon (Bell et al. 1993; Chee-Sanford et al. 2006; Davis 2007).

The contrasting changes we observe in germination parameters under poorly draining and free-draining soils reveal the importance of examining the interaction between nutrient deposition and soil properties on germination and other life history stages of plants. Few previous studies have investigated the combined effects of nutrient addition and soil drainage on germination, and the reasons underlying the patterns we observe in this study remain unclear. Given that added nutrients may persist for longer in poorly drained soils (Howarth et al. 1996; Sims et al. 1998), we suggest two potential mechanisms for differences in germination response as a function of soil drainage. These include (1) direct toxicity of nutrients leading to seed mortality and (2) a temporary arrest of germination due to unfavourable conditions in the presence of nutrients. In poorly draining soils, added nutrients could have persisted in the germination medium for the duration of the experiment, with some losses through volatilisation. This chronic exposure to nutrients may have caused seed mortality through toxicity as described in other studies (e.g. Bremner 1995), resulting in lower germination proportions. In free-draining soils, the lack of a response in germination proportion may stem from a reduction in exposure duration of seeds to the added nutrients as a result of their rapid depletion from the medium through leaching, coupled with volatilisation. On the other hand, the observed trends of delayed germination in nutrient-amended treatments in free-draining soils, which were not manifested in undrained soils, suggest that nutrient addition may result in unfavourable conditions, leading to an arrest of germination. In poorly draining soils, these unfavourable conditions would have persisted for the duration of the experiment, and seed mortality may have occurred indirectly due to other processes, such as decomposition of seeds through fungal or microbial activity (Bell et al. 1993; Chee-Sanford et al. 2006; Davis 2007) in the moist germination medium. In free-draining soils, nutrient addition would have created similar unfavourable conditions for germination only at the initial stages of the experiment. However, more favourable conditions may have been restored over time as nutrients leached out of the germination medium, resulting in delayed germination.

In summary, N-fixers and non-N-fixers may be affected differently by atmospheric N and P deposition, and soil drainage properties may be important in modulating these effects. With N-fixers potentially suffering greater declines in germination proportion in poorly drained soils and a trend of delayed germination in well-drained soils, the hypothesised advantage of N-fixers at the germination stage (Vargas et al. 2015) may be diluted. This could result in a shift in functional dominance of the recruiting tree community in tropical dry forests. However, our current understanding of the mechanisms resulting in these differences, especially those observed between N-fixers and non-N-fixers, is limited and warrants further study. Future work should also consider the role of soil textural differences, and the associated changes in soil drainage and fertility, in mediating germination responses to enhanced nutrient availability. Lastly, whether patterns observed at the seed germination stage persist and are maintained through post-germination life history stages up to reproductive maturity remains unclear, requiring empirical support that takes into consideration competitive interactions as well as disturbance regimes, both of which play important roles in shaping tree communities in this ecosystem.

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