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## Research

### Species-specific root proliferation of tree seedlings in tropical litter: do nutrients matter?

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Litter decomposition mobilizes nutrients that sustain ecosystem productivity, but decomposition by-products may also hamper root proliferation by phytotoxicity. The aim of this study was to assess the litter substrate preferences of tropical tree seedlings in relation to litter chemical traits.

We characterized 44 litter types (11 species at four decomposition ages; 0, 30, 90 and 180 days) for nutrients (N, P, K, Mg, Mn, Na, Fe and Zn) and proximate chemical parameters (cellulose, extractive, lignin and C) and tested the effect of such litter materials on seedling root growth of *Albizia procera*, *Dalbergia sissoo* and *Terminalia arjuna*. *Albizia procera* root growth was inhibited by all litter types and ages, including conspecific materials, while different heterospecific litters had inhibitory or stimulatory effect on *D. sissoo* and *T. arjuna* root growth, compared to the control. Interestingly, inhibitory and stimulatory effects of heterospecific litters significantly changed with litter age, although with no clear-cut pattern among target species and litter species and age, while conspecific litters consistently inhibited root growth when aged, but not when fresh. Litter nutrient, extractive, C, cellulose and lignin showed no consistent association with root growth of tested plants. *Albizia procera* root growth was positively associated with Na content and N:P ratio. *Dalbergia sissoo* root growth was positively associated to C:N and lignin:N ratios, and negatively to K, Na and Zn content. Finally, *T. arjuna* root was positively associated to cellulose and N:P ratio, but negatively to extractive. We conclude that studied nutrient, cellulose and lignin do not consistently explain the species-specific response of root of tree seedlings to decomposing litter.

Keywords: litter decomposition, litter traits, nutrients, root proliferation, tree coexistence

#### Introduction

In tropical forests, high litter fall and decomposition rates are the most important drivers of nutrient cycling (Vitousek and Sanford 1986, Brearley et al. 2003). Several factors, such as plant–soil feedbacks, forest clearance and fragmentation can create spatial and temporal heterogeneity in the forest floor (Jackson and Caldwell 1993,



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Burghouts et al. 1994, Hendriks et al. 2015). Immediately after leaf shedding, litter is attacked by a range of decomposer guilds depending on the wide variation in litter quality among co-occurring species that, in turn, create a physically and chemically heterogeneous environment in the litter layer (Hättenschwiler et al. 2005). Such heterogeneity may largely affect the regeneration niches of tree species, facilitating or inhibiting species recruitment in tropical and temperate forests (Grubb 1977, Brearley et al. 2003).

Studies have reported that litter can affect seed establishment and seedling growth through different mechanisms, eventually limiting or promoting species coexistence (Facelli 1994, Holmgren et al. 1997, Donath and Eckstein 2008, Koorem et al. 2011). In fact, litter can affect physical conditions of forest floor such as temperature, moisture, light quantity and quality (Facelli and Pickett 1991, Holmgren et al. 1997), can create mechanical barriers to seedling emergence (Donath and Eckstein 2008, Ruprecht and Szabo 2012) or can modulate seedling predation activity (Facelli 1994). Decomposing litter can also favor or hamper seedling establishment by releasing essential mineral nutrients (Vitousek and Sanford 1986) or inhibitory allelochemicals during the decay process (Bonanomi et al. 2006, Ruprecht et al. 2010, Koorem et al. 2011).

Nutrient limitation to plant primary productivity is widespread in terrestrial ecosystems, especially in relatively unfertile lowland tropics. Individually or combined, nitrogen (N), phosphorus (P) and potassium (K) are the most common limiting elements (Cuevas and Medina 1988, Vitousek et al. 2010, Wright et al. 2011), although with different behavior. Indeed, N tends to limit plant productivity in young soils, while in ancient soils P becomes increasingly limiting as gradually leached out (Lambers et al. 2008). In a comparative analysis in Amazonian forests of contrasting soil types, Cuevas and Medina (1988) found marked fine root growth stimulation by addition of N in Tall Caatinga forest, of P in Tierra Firme forest and of both nutrients in Bana forests. Consistently, a global meta-analysis comprising 173 studies with independent or factorial manipulations of soil N and P content demonstrated that these two nutrients co-limit terrestrial plant productivity (Elser et al. 2007). As far as K limitation is concerned, Tripler et al. (2006) showed consistent indications of the K limiting effect on the growth rate of forest tree species from several ecosystem types, with watershed K content responsive to disturbance in tropical forest. Furthermore, in Amazonian forest ecosystems with contrasting soil types, particularly the Tierra Firme forests, tree productivity can also be affected by Ca and Mg limitation (Cuevas and Medina 1988), suggesting that other nutrients than N, P and K may play a limiting role in lowland tropical forests (Wright et al. 2011). Finally, a recent study by Kaspari et al. (2014) revealed that in tropical forest exogenously Na application promote fungal and insect activity, with positive effect on leaf and wood decomposition rate.

The magnitude and direction of litter effects can vary in relation to the chemical composition of plant material that,

in turn, depends on litter functional and chemical traits and changes over decomposition time (Lopez-Iglesias et al. 2014, Meiners 2014). In general, two non-mutually exclusive hypotheses have been proposed to explain the inhibitory effects of decaying litter on plant growth and root proliferation. First, nitrogen immobilization by microbial competition (Michelsen et al. 1999) and phytotoxicity by labile organic compounds released during the decomposition process (Inderjit 2005, Bonanomi et al. 2006). N immobilization specifically occurs under N resource limitation, i.e. when mineral N availability is low or when C:N ratio of decaying litter is higher than 30–35, which fosters resource competition between saprophytic microbes and plants (Hodge et al. 2000, Dorrepaal et al. 2007). On the other hand, litter phytotoxic effects can be relevant in field conditions where inhibitory compounds are particularly abundant, such as short-chain organic acids (e.g. acetate, propionate and butyrate) (Armstrong and Armstrong 1999), tannins (Kraus et al. 2003), phenols (Blum et al. 1999) and extracellular DNA (Mazzoleni et al. 2015), released during microbial decomposition. However, during litter decay process, such compounds undergo several physical, chemical and biological transformations that can either increase or decrease their inhibitory effects (An et al. 2001). Also there exists variation in relation to species-specific plant sensitivity to inhibitory compounds (Mazzoleni et al. 2015). In general, the most severe inhibitory effects can be detected at early decomposition stages, followed by a phytotoxicity decline (Jäderlund et al. 1996, Bonanomi et al. 2006), due to rapid degradation of most allelochemicals into non-toxic molecules (Blum et al. 1999, Schmidt and Lipson 2004, Mazzoleni et al. 2015).

During seedling establishment in forests, early root growth and proliferation often occur in the litter layer. Few studies have investigated whether and how such processes are affected by litter type, in terms of litter species identity and decomposition stage (Coq et al. 2012, Lopez-Iglesias et al. 2014, Bonanomi et al. 2017). In this study, we combine litter characterization by proximate analyses, a widely used technique for compositional analysis of C pools (i.e. extractive C, cellulose and lignin) based on acid hydrolysis (Melillo et al. 1989, Preston and Trofymow 2015), and nutrient concentrations (i.e. N, P, K, Mg, Mn, Na, Fe and Zn) with a multi-species seedlings root growth test. In particular, we test the effect of 44 litter types spanning a wide range of biochemical quality on root growth of three target plant species. We expect a species-specific, variable effect of litter on root growth, shifting from inhibitory to promoting at increasing litter age. Specifically, root growth is expected to be stimulated by high quality litter such as *Toona ciliata*, having high N, P and K concentration, whereas inhibited by the low quality litter like *Shorea robusta* with high C:N ratio and lignin content. Moreover, we explicitly hypothesize that root growth is positively associated to litter nutrient concentrations, especially for N, P and K that are considered the most important limiting nutrients in tropical forests (Vitousek 1984, Harrington et al. 2001,

Wright et al. 2011, Preston and Trofymow 2015), and negatively to litter C:N and lignin:N ratios, as related to increasing microbial competition for N sources.

## Material and methods

### Plant litter collection and decomposition experiment

Plant litter materials were obtained from a previous decomposition experiment focused on mass loss and chemical changes (Sarker et al. 2019a), whose procedure is hereby briefly described. Eleven tropical tree species with different leaf traits representing a wide range of litter chemical quality were selected from artificially grown plantation located in Manikganj district (23°51'0"N, 90°0'36"E, central Bangladesh): four evergreen (*Anthocephallus chinensis*, *Artocarpus heterophyllus*, *Mangifera indica*, *Syzygium cumini*), three evergreen to deciduous (*Ficus benghalensis*, *Shorea robusta*, *Terminalia arjuna*) and four deciduous species (*Albizia procera*, *Dalbergia sissoo*, *Lagerstroemia speciosa*, *Toona ciliata*). Freshly abscised leaves were collected (no. of plants > 20) when most of the litter fall occurs by means of nets placed under the canopy, dried (40°C in a ventilated chamber until constant weight was reached) and stored afterwards at room temperature. Litter materials were then transported to Italy for the decomposition experiment, chemical analysis and seedling root proliferation.

The decomposition experiment lasted for 180 days and was carried out under controlled optimal temperature (18 ± 2°C night and 24 ± 2°C day) and water availability (watered every seven days with distilled water), following the litter-bag method (Berg and McClaugherty 2008). The experiment produced 44 litter materials, either undecomposed (thereafter indicated as 0 days) or decomposed for 30, 90 and 180 days. Chemical quality of the 44 litter materials is reported elsewhere in Sarker et al. (2019a) and was used here as a reference dataset (Supplementary material Appendix 1 Table A1). Briefly, all litter samples were analyzed in triplicate and data include nutrient concentration (i.e. N, P, Mg, Mn, Na, Fe and Zn) as well as proximate chemical features (i.e. extractive C, cellulose and lignin contents) that were quantified as acid hydrolysable fraction and acid unhydrolysable materials, respectively, according to Gessner (2005).

### Seed collection and germination

Seeds of three tropical tree species, *A. procera*, *D. sissoo* and *T. arjuna*, were used in the bioassays. Seeds were collected from the same plant used for litter collection and, once at the laboratory, spread over a 3 mm-thick layer of absorbing paper, placed on the bottom of large plastic trays (30 cm deep, 100 cm for each side), where no soil was used. Distilled water was sprayed over the seeds to facilitate germination. Afterwards, the trays were covered and kept in a growth chamber at controlled temperature and moisture conditions, same as described above.

### Seedling root proliferation in polyethylene bags

The root proliferation experiment was carried out in polyethylene bags (PEB) on *A. procera*, *D. sissoo* and *T. arjuna* seedlings, in order to assess the capability of seedling roots to proliferate and exploit the 44 litter types. PEBs were prepared according to Eizenberg et al. (2017) and modified for this study. In detail, 0.5 g of dried and pulverized litter was uniformly spread over a sheet (10 × 22 cm) of glass fiber filter paper. The litter application rate, equivalent to 22.5 g m<sup>-2</sup>, falls within the litter fall range observed in natural ecosystems. Two young seedlings (radicle length: 3–5 mm) were mounted onto untreated control and each prepared filter paper (Supplementary material Appendix 1 Fig. A1). PEBs containing seedlings were placed in a growth chamber at controlled temperature (18 ± 2°C night and 24 ± 2°C day) and moisture conditions and grown for 21 days. Forty-five triplicated treatments were considered, including the application of dry and powdered litter of the 11 plant litter species at the four decomposition stages, and the negative control treated with distilled water. At the end of the experiment, a total of 810 seedlings were analyzed (3 target species × 45 treatments × 3 replicated PEBs × 2 seedlings per PEB). Pictures of seedling roots were taken and total root lengths were measured by image analyzer software (LUCIA, Laboratory Imaging ver. 4.51).

### Statistical analyses

Root length was expressed for each target species and treatment as percentage of the untreated control (i.e. control value = 100). The effects of treatments on *A. procera*, *D. sissoo* and *T. arjuna* root length were assessed by two-ways ANOVA for each target species, including litter age (4 levels) and litter species identity (11 levels) as fixed factors, and their interaction. Pair-wise differences between treatments were tested for statistical significance using Duncan's post hoc test. Pearson's correlation analysis was used to assess the relationships, at each litter age, between litter chemistry (i.e. all nutrients, extractive C, cellulose, lignin, C:N, C:P, N:P, lignin:N and lignin:P ratios) and root length recorded in the root proliferation experiment.

## Results

### Effects of litter types and ages on root growth of the test plant species

Litter effects on root length were largely determined by litter identity, while age-dependent effects were on average limited, but largely different among target species (Fig. 1). Moreover, age-dependent effects were not consistent across treatments (Supplementary material Appendix 1 Table A2, A3), as indicated by statistically significant interaction in the ANOVA (Table 1). As compared to the control, *Albizia procera* root growth was significantly inhibited by all litter types,

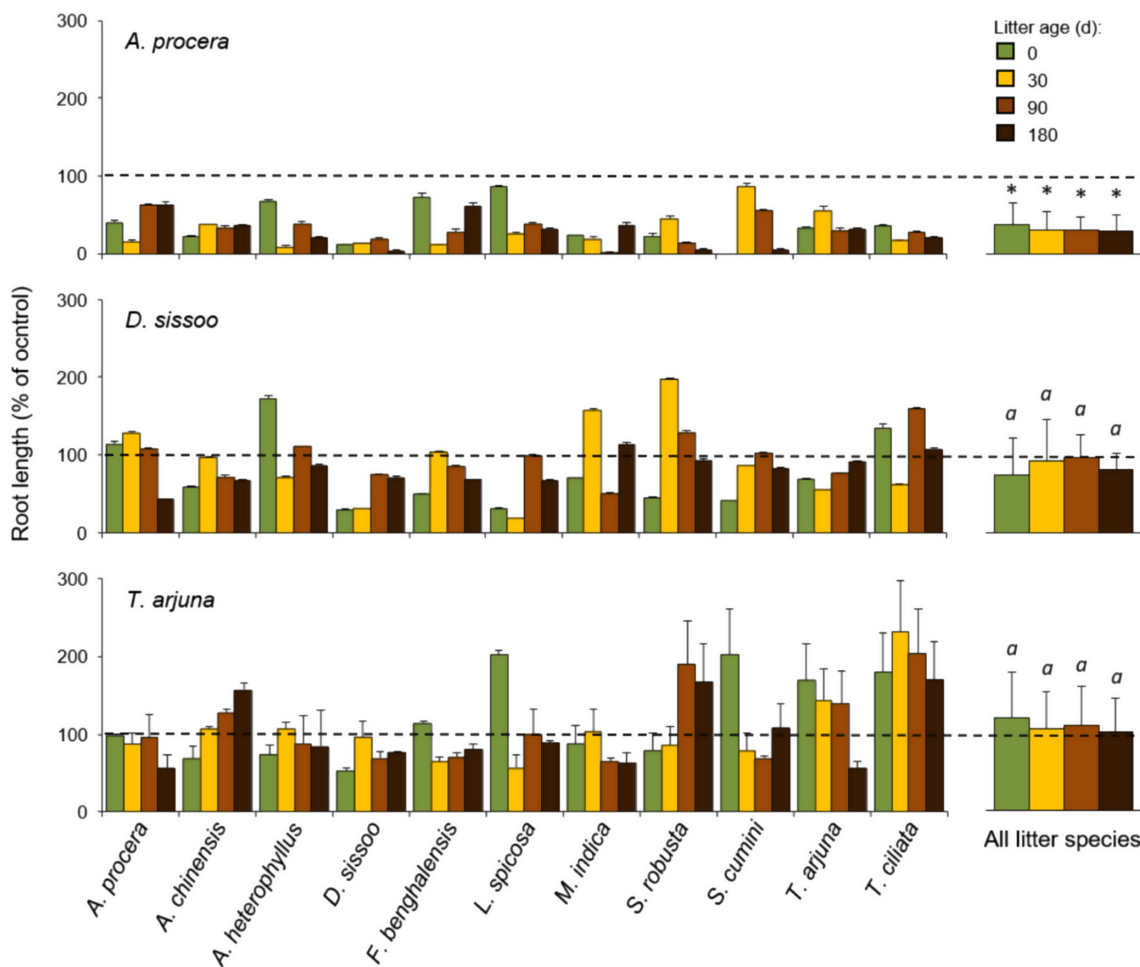


Figure 1. Seedling root length in three tropical tree species (*A. procera*, *D. sissoo*, *T. arjuna*) grown on different litter either undecomposed (0 day) or decomposed for 30, 90 and 180 days, expressed as percentage of the untreated control (black horizontal dashed line). Data refer to mean  $\pm$  SD of three replicates for each target species and litter type (left panels) and mean  $\pm$  SD of data pooled for all litter species (right panels). Different letters above bars indicate significant differences between litter age groups (Duncan test,  $p < 0.05$ ), while asterisks indicate significant differences compared to the control. Statistical support is reported in Table 1 and Supplementary material Appendix 1 Table A2, A3.

including conspecific materials, although the effect magnitude widely varied across litter species and ages (Fig. 1). Differently, *Dalbergia sissoo* root length was stimulated by some litter types, as in the cases of 30 and 90 days old litters of *Shorea robusta* and undecomposed litter of *Artocarpus heterophyllus*, but inhibited by most other litter treatments (Fig. 1). Noteworthy, conspecific litter materials were among the most inhibitory of *D. sissoo* root growth. The response pattern of *T. arjuna* was different from other two target species, with root length inhibited by *M. indica*, *D. sissoo* and *A. procera* litters of different ages, as well as by conspecific 180 days old litter, but unaffected or stimulated by most litter types. Finally, a striking *T. arjuna* root stimulation was observed in presence of aged litter of *S. robusta* and of *Toona ciliata*, the latter independent of litter age (Fig. 1).

### Relationships between litter chemistry and root length

Overall, we found no consistent association between root length and chemical parameters, with only 11 statistically

significant correlations out of 216 cases (Fig. 2). In detail, *A. procera* root length show only two statistically significant correlations with litter Na for fresh litter and N:P ratio when decomposed for 90 days (Fig. 2). *Dalbergia sissoo* root length was positively associated to cellulose, C:N and lignin:N, but negatively with K, Na and Zn concentrations in litters decomposed for 30 days (Fig. 2). Finally, *T. arjuna* root length showed statistically significant positive correlations with N:P ratio and cellulose, but negative with extractive C in case of litter decomposed for 90 days (Fig. 2). Noteworthy, when correlation analysis was carried out pooling litter of different ages no statistically significant correlation emerged (Fig. 2).

### Discussion

Our root proliferation experiment revealed that litter of tropical trees had contrasting effects on seedling root growth, ranging from strong inhibition to stimulation in relation to target species identity, litter type and age (Fig. 1). This is in



Table 1. Summary of two-ways ANOVA for main and interactive effects of litter species (S, eleven levels: *A. procera*, *A. heterophyllus*, *A. chinensis*, *D. sissoo*, *F. benghalensis*, *L. spiciosa*, *M. indica*, *S. robusta*, *S. cumini*, *T. arjuna* and *T. ciliate*) and age (A, four levels: 0, 30, 90 and 180 days) on target species (*A. procera*, *D. sissoo* and *T. arjuna*) root growth. Significant p-values are reported in bold type.

Target species and effect	SS	df	MS	F	p
<i>A. procera</i>					
Litter species (S)	482.962	10	48.296	2.133	<b>0.026</b>
Litter age (A)	51.533	3	17.178	0.759	0.519
S×A	1491.231	30	49.708	2.196	<b>0.001</b>
Error	2988.298	132	22.639		
<i>D. sissoo</i>					
S	505.037	10	50.504	3.533	<b>&lt;0.001</b>
A	69.626	3	23.209	1.623	0.187
S×A	905.079	30	30.169	2.110	<b>0.002</b>
Error	1887.057	132	14.296		
<i>T. arjuna</i>					
S	18244.036	10	1824.404	0.514	0.871
A	1475.382	3	491.794	0.138	0.936
S×A	38695.015	30	1289.834	0.363	0.998
Error	156303.721	44	3552.357		

agreement with the opposite roles that plant litter can have on seed germination and seedling root growth, with a well-recognized beneficial effect as nutrient source (Vitousek and Sanford 1986, Brearley et al. 2003) but also several studies reporting litter inhibitory effects (Rice 1984, Singh et al. 1999, Zhang et al. 2010). In this regards, three studies based on 21 (Dorrepaal et al. 2007), 64 (Lopez-Iglesias et al. 2014) and 65 (Meiners 2014) plant litter types reported a wide spread inhibitory effect of decaying plant residues, with their magnitudes depending on plant functional type and litter decomposition stage. Most of previous studies agreed that undecomposed plant litter has a major inhibitory effect on plant growth but, as decomposition proceeds, litter becomes progressively more suitable for plant growth (Bonanomi et al. 2017).

However, our present findings, showing an idiosyncratic response of root proliferation during litter decomposition, challenge such generalization. In fact, *Albizia procera* root proliferation was inhibited by all litter types at all ages, including conspecific materials, while *Terminalia arjuna* and *Dalbergia sissoo* root growth was either inhibited or stimulated by different litter types (Fig. 1). More specifically, the root growth of *D. sissoo* was stimulated by undecomposed litter of *Artocarpus heterophyllus* and *T. ciliata*, but not from aged residues of the same species. Moreover, root growth of *T. arjuna* was largely stimulated by undecomposed leaf litter of *Lagerstroemia spiciosa* and *Syzygium cumini*, but inhibited by aged litter of these species. Our results highlight that the response of tropical tree seeds to litter is highly species-specific, showing variable and apparently unpredictable behavior. In this context, it could be argued that annual or short-lived species, often used as bioassay because of their sensitivity to organic chemicals, show similar, predictable

trends of root growth in relation to litter age. For instance, in a recent bioassay with 36 litter types tested on 14 plant species, Bonanomi et al. (2017) reported a higher sensitivity to chemicals released from fresh litter for annuals and small-seeded seedlings, as compared to that of large-seeded tree species. Noteworthy, the most sensitive species to fresh litter was *Arabidopsis thaliana*, a short-lived model plant, followed by several annual crops (i.e. *Lycopersicon esculentum*, *Trifolium repens* and *Lepidium sativum*). Concerning tropical tree species, Brearley et al. (2003) reported that litter addition in pot promoted seedling growth of *H. nervosa*, *D. lanceolata* and *P. tomentella*, likely by improving root proliferation and uptake of essential nutrients. Taken together, these evidences suggest that inhibition of root growth by fresh undecomposed litter, as a general phenomenon, does not fully apply to seedlings of tropical tree species.

The molecular and physiological bases for the limited sensitivity of tree seedlings to fresh litter, compared to annual and crop plants, is unknown. Here, we hypothesize that tree species typical of tropical forests, hence continuously exposed to high litter input and rapid turnover, could have adapted to such conditions evolving, among many others, the capability to forage and exploit fresh litter. On the other hand, it could be argued that plants thriving on mineral soil or sediment are more sensitive to litter chemical properties. It is worth to mention that in our experiment we have purportedly used pulverized litter materials to minimize possible effects related to litter physical properties, thus focusing on the effect of litter chemical features on tree seedling root growth. However, the physical properties of the litter materials may have a major influence on root growth by interacting with chemical properties in real field conditions.

Our findings raise as further question: why seedling response to litter is species-specific? For instance, we found that *A. procera* root growth was inhibited by all litter types, including conspecific litter. On the contrary, *D. sissoo* and *T. arjuna* were largely stimulated by *T. ciliate* litter. In general terms, root growth over plant litter could be explained by litter nutrient availability or the occurrence and activity of phytotoxic organic compounds. The nutrient immobilization hypothesis states that litter with high C:N and lignin:N ratio would hamper root system development due to low nitrogen availability at the short-term. Our results do not support such hypothesis, since all our litter materials had low C:N ratios and rapidly mineralized N during the decomposition process (Sarker et al. 2019a). Moreover, root growth of all our target species showed inconsistent patterns of association with litter N concentrations and C:N ratios (Fig. 2). On the contrary, for *D. sissoo* we found positive correlation with C:N and lignin:N ratio, suggesting that N is not a limiting factors for this species, at least at intermediate decomposition stages. Therefore, our results indirectly suggest that other causal factors should be considered to explain litter inhibitory effects on root proliferation.

On the other hand, *D. sissoo* and *T. arjuna* showed a strong root proliferation over some litter types, in some

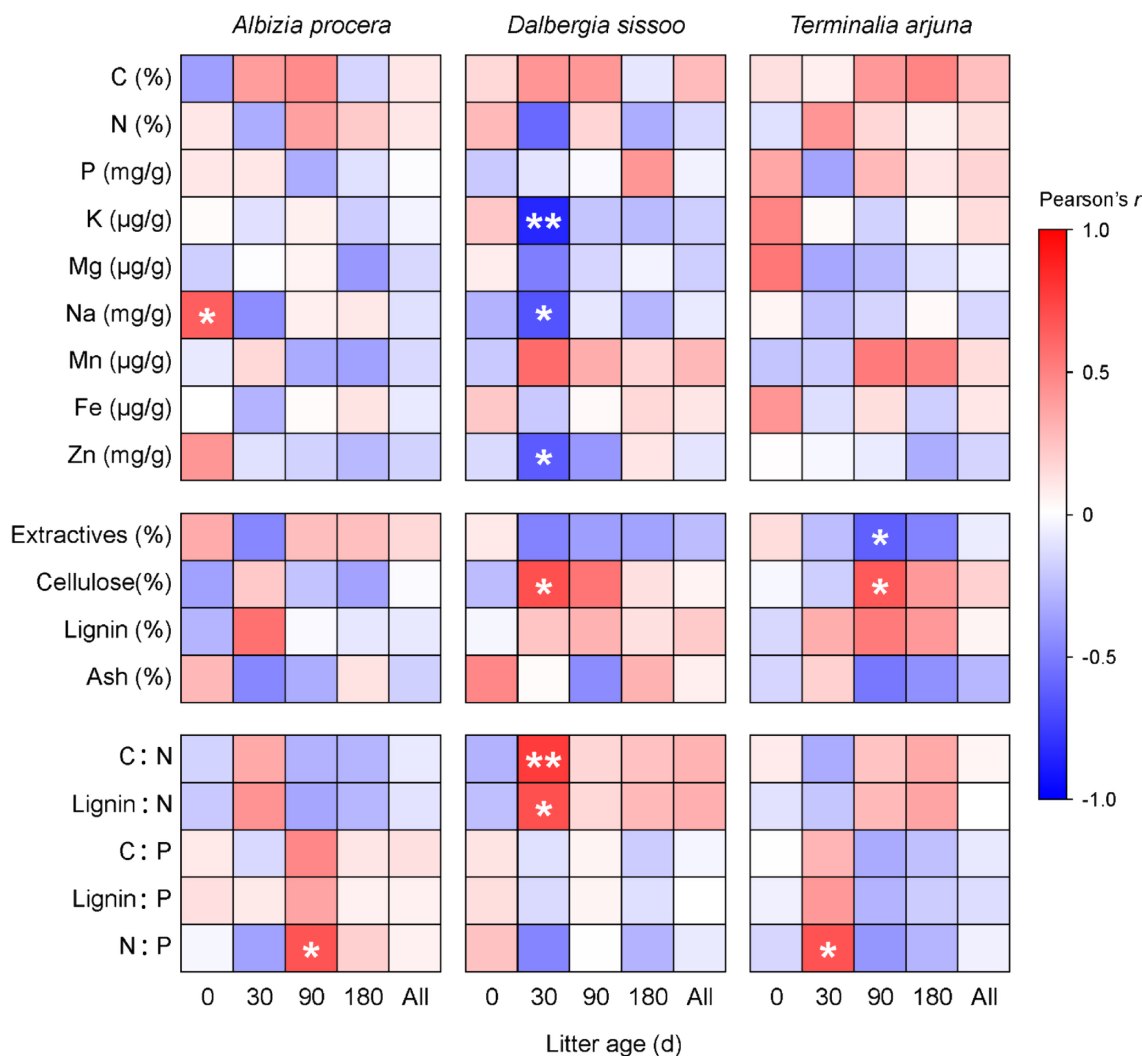


Figure 2. Heatmap showing the association between seedling root length of three tropical tree species (*A. procera*, *D. sissoo*, *T. arjuna*) grown on 11 litter species at four litter ages (0, 30, 90 and 180 days) and litter chemical descriptors. Asterisks indicate significant correlation values (Pearson's  $r$ ; \*\*,  $p < 0.01$ ; \*,  $p < 0.05$ ,  $n = 11$  at each litter age and  $n = 44$  for data pooled for all litter ages), either positive (red) or negative (blue).

cases doubling the growth recorded for the control. It is well known that, during decomposition, litter releases considerable amounts of nutrients that are readily absorbed by seedling roots (Vitousek 1984, Brearley et al. 2003). N, P and K are considered the most important limiting factors for tree growth in tropical forests (Vitousek 1984, Kaspari et al. 2008). Accordingly, a number of studies have shown that the addition of nutrients (i.e. N, P and in some cases K) in inorganic forms increased tree growth both in controlled and field conditions (Nussbaum et al. 1995, Yap et al. 2000). Moreover, Wright et al. (2011) reported that N, P and K can limit tree growth in relatively fertile soils in lowland forest in Panama, with the addition of K particularly decreasing fine-root biomass. Similarly, Sayer et al. (2012) observed changes in root biomass due to K addition in an old-grown lowland tropical forest. In our study, however, we found only few and

weak association of litter N, P and K concentrations with root growth (Fig. 2). Such result apparently contrasts with the well-known ability of plant roots to selectively proliferate into nutrient-rich patches (Robinson 1994, Hodge 2004). However, it could be easily explained considering that N, P and K effects can substantially differ whether provided in mineral or organic forms, so that the concentration of major nutrients, per se, is scarcely predictive of root foraging and proliferation (Vitousek et al. 2010, Wright et al. 2011).

As far as other litter nutrients are concerned, in agreement with earlier findings by Gunatilleke et al. (1997), we observed that root growth of the target plants was unrelated to Mg concentration (Fig. 2). Other nutrients such as Na showed either positive and negative correlation with root length, do not showing the consistent patterns of association recently reported with wood decay rate (Kaspari et al. 2014,

Pennisi 2014). Recently, Sarker et al. (2019a) showed the importance of micro nutrients for litter decomposition, by recording positive correlations between mass loss and concentrations of Fe, Mg, Zn and K in undecomposed litter, which could be due to the microbial redox processes (Silver et al. 2013, Hall et al. 2015), but negative correlation in the case of Mn. These results indicate nutrients (N, P, K, Ca and Na) are good predictors of leaf litter and wood decay rate but cannot properly explain the root proliferation of tree seedlings in tropical forests.

Overall, it is noteworthy that most of nutrient, proximate C pools and their ratios (C:N, C:P, N:P, lignin:N and lignin:P) were largely unrelated to either litter inhibitory and stimulatory effects on root growth. The poor capability of nutrient to predict litter effect on seedling suggests that other factors control the specific response of root to litter. In this scenario, the use of  $^{13}\text{C}$  NMR to describe litter chemistry in the last years greatly improved the current understanding of the effects of litter on soil functioning (Carteni et al. 2018). Bonanomi et al. (2017) tested 14 target plants species, including temperate tree and agricultural species, over 36 litter types and found that organic C functional types better predicted root proliferation than nutrient concentration. In detail, root proliferation was negatively correlated to the content of di-O-alkyl C and O-alkyl C, mainly associated with sugars and carbohydrates, but stimulated by litter rich in methoxyl C that is typical of lignified tissue. Interestingly, in the present work we found a striking stimulation by *T. ciliata* litter that may be attributed to its organic C molecular composition characterized by high methoxyl C, but low in O-alkyl C (Sarker et al. 2019a). Consistently, root stimulation of *Lepidium sativum* was previously reported to be positively associated to methoxyl C content in a study based on 64 litter types (Bonanomi et al. 2011). In this context, we expect that an extensive application of  $^{13}\text{C}$  NMR to tropical litter may contribute to solve the specific but contradictory effect of plant litter on seedling growth and establishment.

## Conclusions

Seedling establishment is a crucial stage for species coexistence in forest ecosystems, and plants are particularly sensitive to litter occurrence, abundance and quality during early ontogenetic stages. Our study, based on multi-species seedling root growth test and 44 tropical litter types, revealed a species-specific response, with root proliferation either promoted or inhibited, depending on litter identity and age. Extensive correlation results indicate that nutrient concentration dynamics do not exhaustively explain seedling foraging preferences for different litter types. Therefore, we suggest that plant litter roles are not limited to acting as a nutrient source, and further processes must be taken into account to fully understand litter implication in plant establishment, growth and coexistence. Specifically, we believe that future studies should integrate litter nutrient availability with litter

carbon quality characterization by  $^{13}\text{C}$  NMR, in order to assess the impact of decomposing materials on root proliferation and seedling establishment.

## Data availability statement

Data are available from the Dryad Digital Repository: <<http://dx.doi.org/10.5061/dryad.bzkh18955>> (Sarker et al. 2019b).

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*Conflict of interest* – All authors declare that they have no conflict of interest.

*Author contribution statement* – TCS and GB conceived and designed the experiments and TCS performed the experiments. TCS, VM, SCP and MI collaborated in image analysis. TCS, GI, ADM and GB performed statistical analyses. TCS, GM, GI and GB wrote the manuscript; other authors provided editorial advice.

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Supplementary material (available online as Appendix oik-06786 at <[www.oikosjournal.org/appendix/oik-06786](http://www.oikosjournal.org/appendix/oik-06786)>).  
Appendix 1.