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Seed polyphenols in a diverse tropical plant community

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Abstract

1. Polyphenols are one of the most common groups of secondary metabolites in plants and thought to play a key role in enhancing plant fitness by protecting plants against enemies. Although enemy-inflicted mortality at the seed stage can be an important regulator of plant populations and a key determinant of community structure, few studies have assessed community-level patterns of polyphenol content in seeds.

2. We describe the distribution of the main seed polyphenol groups across 196 tree and liana species on Barro Colorado Island (Panama) and community-level patterns in two aspects of their biological activity (protein precipitation and oxidative capacity). Taking advantage of substantial variation in morphological and ecological traits in the studied plant community, we test for correlations and trade-offs between seed polyphenols and nonchemical plant traits hypothesised to make plant species more or less likely to invest in polyphenol production.

3. The majority of species have polyphenols in their seeds. The incidence and concentrations of polyphenols were related to a set of nonchemical plant traits. Polyphenols were most likely to be present (and where present, to be expressed in high concentrations) in species with large seeds, short seed dormancy times, low investment in mechanical seed defences, high wood density, high leaf mass per area, tough leaves and slow growth rates.

4. *Synthesis.* Our study reveals a potential trade-off between chemical and mechanical seed defences and shows that plant species that invest in physical defences at later life stages (high wood density and tough leaves) tend not to invest in physical defences of seeds but instead produce secondary metabolites likely to act as seed defences. Overall, our results conform to predictions from the resource availability hypothesis, which states that species in resource-limited environments (such as slow-growing shade-tolerant tree species) will invest more in defences than fast-growing pioneer species.

KEYWORDS
Barro Colorado Island, chemical seed defences, determinants of plant community diversity and structure, macroevolution of plant defences, plant apparency theory, resource availability hypothesis, tannins

1 | INTRODUCTION

The world being green can at least in part be attributed to plant defences (Hartley & Jones, 1997; Murdoch, 1966). Understanding the causes and consequences of variation in the defensive profiles of plant individuals and species has become a key aim of chemical ecologists and ecologists studying plant–enemy interactions. By examining the distribution of defensive traits across the plant phylogeny (Agrawal, Lajeunesse, & Fishbein, 2008; Cacho, Kliebenstein, & Strauss, 2015; Harborne, 1977; Johnson, Ives, Ahern, & Salminen, 2014; Mole, 1993) or across plant species within a community (Coley, 1983; Kraft et al., 2015; Schuldt et al., 2012), ecologists seek insights into the factors driving their evolution and to learn more about their likely roles in contemporary ecological processes (e.g. Cárdenas, Valencia, Kraft, Argoti, & Dangles, 2014; Carmona, Lajeunesse, & Johnson, 2011; Kursar et al., 2009; Loranger et al., 2012). By assessing correlations and trade-offs between different types of defensive traits or between defensive traits and other types of plant traits (Agrawal, 2007; Agrawal & Fishbein, 2006; Johnson et al., 2014; Kursar & Coley, 2003), it may also be possible to gain insights into the evolutionary ecology of plant defences. At the same time, a framework for predicting the susceptibility of individual plant species to enemy attack based on sets of more easily measurable plant traits can be created (see e.g. Kraft et al., 2015).
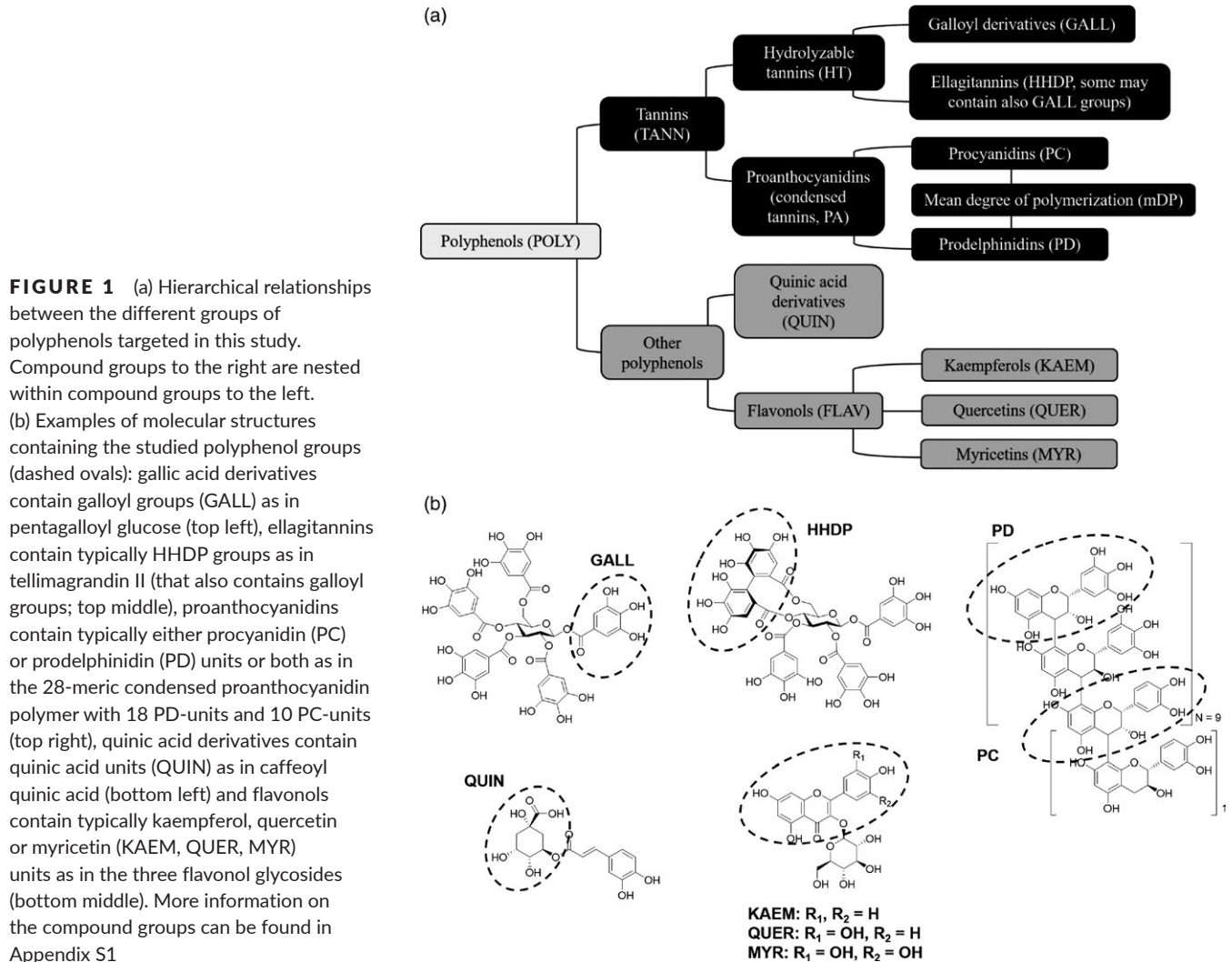
Since plant defences can be costly to produce (Strauss, Rudgers, Lau, & Irwin, 2002), the pay-off for investment in defences may sometimes be context-dependent. For example, where the risk of enemy attack is low or unpredictable, plants might benefit from allocating their resources to growth or reproduction rather than to defences (Bazzaz, Chiariello, Coley, & Pitelka, 1987) and rely on induced defences that allow them to switch on defences following enemy attack (e.g. Karban & Myers, 1989). Although some defence mechanisms are plastic, the interspecific variation in constitutive plant defences is often considerable. A rich body of theory predicts under what circumstances plants are most likely to invest heavily in constitutive defences (e.g. Agrawal, 2007; Bazzaz et al., 1987; Coley, Bryant, & Chapin, 1985; Feeny, 1976; Herms & Mattson, 1992). For example, it has been suggested that the degree to which a plant species invests in defences will depend on its 'apparency', that is, its proneness to discovery and attack by enemies (Feeny, 1976; but see Smilanich, Fincher, & Dyer, 2016). Parallel lines of reasoning suggest that investment in plant defences may also depend on the general life history strategy of the species or on the growth environment. For example, slow-growing species might invest more in defences than fast-growing species, trees might invest more in defences than lianas, and plants adapted to grow in resource poor environments might invest more in defences than plants adapted to resource rich environments (Asner & Martin, 2012; Coley et al., 1985; Janzen, 1974).

A remarkable bias in the current literature on macroevolutionary patterns of plant defences is the strong focus on leaf defences in adult plants. Although investment in defences can vary with ontogeny (Boege & Marquis, 2005; Elger, Lemoine, Fenner, & Hanley, 2009; Kariñho-Betancourt, Agrawal, Halitschke, & Núñez-Farfán, 2015) and

between different plant parts (Beckman, 2013; Çırak, Radušienė, Janulis, & Ivanauskas, 2007; McKey, 1979; Tuominen, Toivonen, Mutikainen, & Salminen, 2013) and the impact of enemies on the evolution of plant defences might differ between different stages of the plant life cycle (Boege & Marquis, 2005; Janzen, 1969), multi-species studies assessing the distribution of defensive traits in plants of different age cohorts and in nonfoliar plant tissues are scarce. Collating and analysing data on community-level patterns of defence expression in the early stages of the plant life cycle could yield new insights into the role of plant defences in ecological processes of key importance. This is particularly true given that the seed and seedling stages can form important bottlenecks in the regeneration dynamics of plants (Fenner & Thompson, 2005; Grubb, 1977; Leck, Parker, & Simpson, 2008) and the realisation that ecological processes involving the early stages of the plant life cycle are important in community assembly (Green, Harms, & Connell, 2014). If data on seed defences can be combined with trait information collected at later ontogenetic stages, there is also an opportunity to assess the extent to which defence traits expressed at different stages of the plant life cycle tend to covary (see e.g. Barton & Boege, 2017).

In this paper we use both ecological and chemical expertise to describe patterns of chemical seed defences in the well-studied woody plant community (trees, shrubs and lianas) on Barro Colorado Island (BCI), Panama. We focus on one group of chemical compounds that is widely assumed to protect plants against enemies: the polyphenols, with special emphasis on the most common subgroups of tannins, that is, hydrolysable tannins and proanthocyanidins (Barbehenn & Constabel, 2011; Bernays, Cooper-Driver, & Bilgener, 1989; Salminen & Karonen, 2011; Waterman & Mole, 1994). We also measure two tannin-related bioactivities: protein precipitation capacity and oxidative capacity. The former is potentially effective at acidic to neutral pH as found in guts of mammalian herbivores (Appel, 1993; Barbehenn & Constabel, 2011; Beasley, Koltz, Lambert, Fierner, & Dunn, 2015) and the latter is potentially effective at alkaline pH as found in midguts of insect herbivores, especially lepidopteran larvae (Appel, 1993; Dow, 1992; Harrison, 2001). To gain a detailed picture of the concentrations of the most common polyphenol groups typically encountered in green parts of plants, we quantify eight common functional polyphenol groups (Figure 1) including four types of tannins (gallic acid derivatives, ellagitannins, procyanidins, prodelphinidins), three types of flavonols (kaempferol, quercetin and myricetin glycosides) and quinic acid derivatives (containing typically quinic acid esters of caffeic, coumaric and gallic acids).

We have three goals: (1) to describe patterns of polyphenol distribution, protein precipitation capacity and oxidative capacity in seeds within the woody plant community on BCI, (2) to relate polyphenol distributions to seed and plant traits hypothesised to place plant species under higher or lower pressure to evolve or retain a high investment in polyphenols (see below), and (3) to assess whether investment in chemical seed defences tends to be related to investment in other types of seed defences and defences at later stages in the life cycle. While polyphenols can have multiple ecological roles (e.g. Waterman

(a)

FIGURE 1 (a) Hierarchical relationships between the different groups of polyphenols targeted in this study. Compound groups to the right are nested within compound groups to the left. (b) Examples of molecular structures containing the studied polyphenol groups (dashed ovals): gallic acid derivatives contain galloyl groups (GALL) as in pentagalloyl glucose (top left), ellagitannins contain typically HHDP groups as in tellimagrandin II (that also contains galloyl groups; top middle), proanthocyanidins contain typically either procyanidin (PC) or prodelphinidin (PD) units or both as in the 28-meric condensed proanthocyanidin polymer with 18 PD-units and 10 PC-units (top right), quinic acid derivatives contain quinic acid units (QUIN) as in caffeoyl quinic acid (bottom left) and flavonols contain typically kaempferol, quercetin or myricetin (KAEM, QUER, MYR) units as in the three flavonol glycosides (bottom middle). More information on the compound groups can be found in Appendix S1

& Mole, 1994), our study focuses specifically on their role in defending plants from enemies; hence our choice to measure subgroups of polyphenols that have been shown to be associated with plant defence in other contexts (e.g. Agrawal, Hastings, Johnson, Maron, & Salminen, 2012; Feeny, 1970; Haukioja, 2003; McArt, Halitschke, Salminen, & Thaler, 2013; Roslin & Salminen, 2008).

Following the widely accepted assumptions that polyphenols contribute to protecting plants from enemies such as seed predators and pathogens (e.g. Tellez, Rojas, & Van Bael, 2016; Waterman & Mole, 1994) and that polyphenol production incurs a cost to the plant (Strauss et al., 2002), we predict that natural selection will have resulted in nonrandom patterns of polyphenol distribution across species in the plant community. Inspired by plant apparency theory (Feeny, 1976), which predicts that plant species with traits that make them particularly prone to enemy attack will invest heavily in defences, we make the following predictions:

1. Positive relationship between *maximum tree height* and polyphenol content, because the larger the adult tree, the larger and more obvious the seed crop is likely to be to pre-dispersal seed predators and other enemies.

2. Positive association between polyphenol content and the *typical length of seed dormancy*, because the longer the time lag between seed production and germination, the more time there will be for exposure to post-dispersal seed enemies.

Inspired by resource availability theory (Coley et al., 1985), which predicts that the costs of enemy attack will be highest for species growing in resource-limited environments, we predict:

3. Negative relationship between *relative growth rates* (RGRs) and polyphenol content, because slow-growing species will invest more in polyphenols than fast-growing species (cf. Coley, 1983).

Inspired by hypothesised resource allocation trade-offs (e.g. Bazzaz et al., 1987; Steward & Keeler, 1988), we make the following predictions:

4. Positive relationship between *seed size* and polyphenol content, because when reproductive potential is limited to a few large seeds they must be well defended (cf. Smith & Fretwell, 1974).

5. Negative relationship between investment in *mechanical seed defences* and polyphenol production, because investment in multiple

types of defences may be costly (e.g. Koricheva, Nykänen, & Gianoli, 2004; Moles et al., 2013; Steward & Keeler, 1988).

Finally, we predict:

6. Positive relationship between seed polyphenol content and *defences expressed in other types of plant tissues* later in the life cycle such as wood density and traits associated with leaf toughness, if species tend to consistently invest heavily in defences (Agrawal, 2011) regardless of tissue type and throughout the life cycle (but see e.g. Barton & Boege, 2017).

7. Higher polyphenol investment in *trees* than in *lianas*, because lianas are widely believed to be characterised by fast life history strategies that maximise resource uptake and growth while minimising allocation to defence (Asner & Martin, 2012; but see Gilbert, Wright, Muller-Landau, Kitajima, & Hernandéz, 2006).

2 | MATERIALS AND METHODS

2.1 | Study site and study system

Our study targets the plant community of BCI in central Panama (latitude 9.1543, longitude −79.8461). BCI is approximately 15 km^2 in size and was isolated from the surrounding mainland in 1913 when Gatun Lake was formed during the construction of the Panama Canal. Annual rainfall averages 2,600 mm with a pronounced dry season between December and April/May. The yearly average temperature is 25.8°C (Smithsonian Tropical Research Institute 2017). The woody plant community of BCI is exceptionally well studied and the ecology of many of the tree and liana species is well known (e.g. Hubbell et al., 1999; Schnitzer et al., 2012), making the site particularly well suited for our study. The reproductive ecology of trees and lianas in a permanent 50 ha forest dynamics plot has been addressed through a long term study yielding an unprecedented dataset on patterns of flower and seed production for a large number of species (e.g. Harms, Wright, Calderon, Hernandez, & Herre, 2000; Wright, Muller-Landau, Calderón, & Hernandéz, 2005). While many traits have been characterised for many plant species (e.g. Coley, 1983; Daws, Garwood, & Pritchard, 2005; Garwood, 1983; Sautu, Baskin, Baskin, & Condit, 2006; Sautu, Baskin, Baskin, Deago, & Condit, 2007; Westbrook et al., 2011; Wright et al., 2005, 2010), the polyphenol content of seeds has so far not been investigated.

2.2 | Collecting and preparing seeds for chemical analyses

Seeds and fruits of a large number of woody plant species and a smaller number of nonwoody vines and epiphytes were collected on BCI between July 2010 and December 2013 as part of a community-level study of insect seed predation (S. Gripenberg, unpublished data). BCI trails were walked regularly and freshly fallen fruits and seeds were collected. A subset of the mature fruits and seeds collected

during the peak seasons of fruit production in 2011 and 2013 was set aside for phytochemical analyses. All fruit pulp was removed and the seeds were counted, placed in paper envelopes, and freeze-dried to constant weight for a minimum of 3 days, after which the samples were stored in airtight plastic ziplock bags in a freezer until exported under permit from the *Autoridad Nacional del Ambiente* (ANAM).

Each 'sample' consisted of seeds (including embryo, endosperm and testa) of a given species collected in a given location. All seeds within a sample were collected on the same day. Since seeds were typically encountered under fruiting individuals, it is likely that most seeds within a sample shared the same maternal source. Since seed mass varies by five orders of magnitude among woody species on BCI (Wright et al., 2010), standardising the number of seeds per sample was not feasible. To maximise the number of species in our dataset, we therefore allowed the quantity of seeds to vary between species and samples, ensuring that the volume of each sample was large enough to allow their polyphenol chemistry to be analysed (we aimed for a minimum sample dry weight of 50 mg). While our aim was to collect several samples per species, many plant species are rare in the forest and encountering multiple fruiting individuals was not possible with available resources. Consequently, 69 of the species in our dataset (35%) are represented by one single sample. Altogether, 572 samples representing 196 plant species (one of these species could only be identified to genus level), 149 genera, 54 plant families and 26 orders were analysed. For 174 samples (30%) it was not possible to obtain fully accurate quantitative measurements of polyphenol content since the samples were oily even after freeze-drying. Thus the quantitative polyphenol contents of these samples were expressed against the weights that included the oily component. These samples were only used in analyses on qualitative patterns of polyphenol distribution. The polyphenol content was accurately measured for the remaining 398 samples, which included 144 species.

2.3 | Chemical analyses

Our chemical analyses focused on assessing the incidence (i.e. presence or absence) and concentration of polyphenols and two properties relevant to the role of polyphenols in plant defence: protein precipitation and oxidative capacity. Different types of polyphenols can have different biological properties (Quideau, Deffieux, Douat-Casassus, & Pouységu, 2011; Salminen & Karonen, 2011). Because of this, we assessed the incidence and concentration of a number of subgroups of polyphenols by focusing on the biologically relevant functional units present in tannins and other polyphenols with the help of selective and sensitive ultraperformance liquid chromatography mass spectrometry (UPLC-MS/MS) methods recently developed by Engström et al. (2014) and Engström, Pälijärvi, and Salminen (2015) (Figure S1). In addition, we assessed the overall incidence and concentration of all polyphenols in the samples by the less specific Folin-Ciocalteu assay. Altogether, 16 chemical variables (some nested within others) were assessed for each sample (Figure 1, Appendix S1).

The analyses were conducted in the laboratory of the Natural Chemistry Research Group at the University of Turku, Finland. Once

the samples were obtained from the field site, they were freeze-dried a second time and ground into fine powder. We extracted 20 mg of the powder for 3 hr with 1.4 ml of acetone/water (80:20, v/v) twice. After evaporation of the acetone from the combined extract, the water-phase was freeze-dried and dissolved into 1 ml of MilliQ purified water. This extract was filtered via 0.20 μm PTFE filters, diluted 5× by MilliQ purified water and analysed by UPLC-MS/MS as described in Engström et al. (2014, 2015) and Malisch et al. (2015). The nondiluted extract was used for the total phenolic and oxidative activity measurements as described in Salminen and Karonen (2011). For the radial diffusion assay (used to assess the protein precipitation capacity of the extracts; Hagerman, 1987) the nondiluted extract needed to be 2× concentrated via freeze-drying and redissolving into water. 24 μl of the extract was applied on three wells punched onto a Petri dish filled with bovine serum albumin (BSA)-agar gel. The Petri dishes were covered by parafilm and incubated at 30°C for 72 hr to form reproducible rings with tannins and BSA. For details on the methods see Appendix S1.

2.4 | Nonchemical plant trait data and plant phylogeny

To address goals 2 and 3, we took advantage of the natural variation in ecological and morphological traits exhibited by plant species on BCI. The nonchemical plant trait data used in our analyses were obtained from a variety of published and unpublished sources. *Maximum height* (H_{max}; expressed in m; prediction 1) was measured following methods described in Wright et al. (2010). *Mean length of seed dormancy* (days from sowing until germination; prediction 2) was extracted from Garwood (1983) and Sautu et al. (2006) and supplemented with a previously unpublished dataset collected by N. Garwood from 1985 to 1989. All three sources used methods similar to those described in Garwood (1983). For species for which the mean length of seed dormancy was assessed for multiple batches of seeds, we calculated an overall mean weighting by the number of seeds in each batch.

The *RGR* (cm per year; prediction 3) of saplings and trees was assessed using methods described in Wright et al. (2010). In the context of this study, we used mean RGRs. Since growth-survival relationships can differ between growth forms (Wright et al., 2010), we restrict our analyses of relationships between RGR and chemical variables to canopy trees only.

We dissected diaspores to determine *seed mass* (grams; prediction 4) and *protective tissue content* (proportion of diaspore mass comprised by protective tissue; prediction 5). Fresh diaspores were dissected to separate appendages that enable dispersal (e.g. wings in wind-dispersed species), tissues that encase and protect the seed (testa plus endocarp), and the living seed and its resources (embryo plus endosperm) (Wright et al., 2010; S. J. Wright, unpublished data). The dissected diaspores were oven dried to constant mass at 60°C. Seed mass equals the dry mass of embryo plus endosperm. Protective tissue content equals the dry mass of the testa plus endocarp encasing the seed normalised by the dry mass of the diaspore. For most species, we dissected five seeds collected from five individuals. Sample sizes were slightly lower for some species.

Toughness (J) and peak force (N) quantify the energy or maximum force required to break the diaspore and are relevant to prediction 5 (Fricke & Wright, 2016). There is a strong positive relationship between these variables and diaspore mass due to a physical scaling relationship (Fricke & Wright, 2016). We therefore measured *relative toughness* and *relative peak force* as residuals from the relationship between toughness or peak force and diaspore mass. Fricke and Wright (2016) describe the methods used to measure toughness and peak force.

Wood specific gravity (WSG; g/cm^3; prediction 6) and *leaf mass per area* (LMA; g/m^2; prediction 6) were measured using methods described in Wright et al. (2010). *Lamina fracture toughness* (LamFT; J/m^2; prediction 6) was extracted from the Dryad Digital Repository (data associated with Westbrook et al., 2011). Based on their *growth form*, all woody plant species were classified as either trees or lianas (prediction 7). The full dataset on chemical and nonchemical traits used in this study is provided in Gripenberg et al. (2017).

We used an updated version of the phylogeny published by Kress et al. (2009) to account for non-independence of closely related species in the analyses addressing patterns of association between chemical and nonchemical traits. David Erickson (Department of Botany, Smithsonian Institution, National Museum of Natural History, USA) provided the updated ultrametric phylogeny which includes 548 species sequenced for the same three loci as in Kress et al. (2009). From this phylogeny we removed the species that we did not sample and then we manually added 10 species as described in Appendix S2, which resulted in a 190-species tree.

2.5 | Statistical analyses

To assess the degree of intraspecific variation in polyphenol concentrations (which could, if large, complicate the use of species-specific mean polyphenol concentrations in the community-level analyses), we conducted variance component analyses to quantify the degree of inter- vs. intraspecific variation in the concentrations of each polyphenol group. In these analyses, sample-specific log-transformed polyphenol concentrations were modelled as a function of *species* (a random factor) using the lme4 library in R (Bates, Maechler, Bolker, & Walker, 2015). The analyses were run on a subset of the data that included species with three or more samples. To allow us to include samples with zero concentrations of respective compound group, a small constant (0.001) was added to the concentrations. The proportion of the total variance explained by *species* was taken as the amount of variation occurring at the interspecific level.

Most of our analyses assessing associations between chemical and nonchemical traits were conducted using a two-step approach. In the first step, we examined patterns of incidence of polyphenols (or individual subgroups of polyphenols), protein precipitation and oxidative capacities across species. In the second step, we examined polyphenol concentrations (or concentrations of individual subgroups of polyphenols) or quantitative protein precipitation and oxidative capacities. In the second step, we excluded species in which polyphenols (or particular subgroups of polyphenols), protein precipitation or

oxidative capacity had not been detected. Excluding these species was necessary to achieve normality of model residuals in the analyses addressing quantitative response variables. All statistical analyses were conducted in R 3.2.2 (R Core Team 2015).

We first conducted a series of bivariate analyses to evaluate associations between chemical and nonchemical traits (goals 2 and 3) using statistics that control for phylogenetic relationships. We performed bivariate analyses because we wanted to include all species and many of the nonchemical traits were only available for a subset of species. When the chemical variable was binary (incidence of polyphenols, protein precipitation or oxidative capacities), we used phylogenetic logistic regression (Ives & Garland, 2010), implemented in the *phyloglm* function of the R phylolm package (Ho & Ane, 2014). When the chemical variable was continuous (concentrations of chemical compounds or quantitative measures of protein precipitation and oxidative capacities), we used phylogenetic generalised least squares (PGLS) analyses using packages NLME (Pinheiro, Bates, DebRoy, & Sarkar, 2014) and APE (Paradis, Claude, & Strimmer, 2004). We calculated Pagel's λ (a measure of phylogenetic signal in model residuals) for each PGLS regression using restricted maximum likelihood. We only conducted PGLS regressions for trait combinations represented by ten or more species. To account for the fact that we conducted a large number of analyses, we calculated Benjamini-Hochberg corrected *p*-values (Benjamini & Hochberg, 1995) to control the false discovery rate within each group of tests. We present raw *p*-values alongside the Benjamini–Hochberg corrected ones.

We then evaluated the correlation structure between the wider set of chemical and nonchemical variables using a Principal Coordinates Analysis (PCoA; Gower, 1971). We chose PCoA instead of the more common Principal Components Analysis because PCoA allows inclusion of multiple types of variables (Pavoine, Vallet, Dufour, Gachet, & Daniel, 2009) and can more easily accommodate missing data (Dray & Josse, 2015). We conducted the PCoA for a generalised Gower distance matrix (Pavoine et al., 2009) which was corrected using the Lingoes transformation (Lingoes, 1971) to obtain Euclidean distances. We excluded redundant chemical variables (i.e. polyphenol groups below the tips of the polyphenol hierarchy in Figure 1). To analyse the structure in the trait data, we assessed correlations (Pearson's *r*) between trait values and the two major axes of the PCoA. We log transformed variables with skewed distributions prior to analysis. To aid interpretation of our results, we tested for covariation between explanatory variables by assessing all pairwise nonchemical trait correlations; Table S1.

3 | RESULTS

3.1 | Distribution of seed polyphenols in the BCI plant community

Polyphenols are common and widespread in seeds of BCI plants (Table 1): phenolic compounds and polyphenols were detected in 86% and 76% of species respectively. The most frequently detected subgroup of polyphenols was proanthocyanidins (condensed tannins)

that contained procyanidin subunits, followed by hydrolysable tannins containing galloyl subunits (common to galloyl glucoses, gallotannins and ellagitannins), proanthocyanidins containing prodelphinidin subunits, hydrolysable tannins with hexahydroxydiphenoyl (HHDP) subunits (common to ellagitannins) and polyphenols containing quinic acid moieties (common to, for example, caffeoyl and coumaroyl quinic acids). The three flavonol groups (myricetin, kaempferol and quercetin derivatives) were found in a relatively small number of species (Table 1). Protein precipitation and oxidative capacity were detected in 25% and 35% of the species respectively.

Across the 144 species that could be used for assessment of polyphenol concentrations in seeds (i.e. species without oils that prevented complete drying of seeds), the mean concentrations per species differed substantially among the studied subgroups of polyphenols (Table 1). In general, the polyphenol subgroups that were present in a large proportion of species also tended to be present in high mean concentrations, the exception being the hydrolysable tannins with HHDP subunits that were present in a relatively small proportion of the species but in relatively high concentrations (Table 1).

Two thirds or more of the variation in polyphenol concentrations occurred among species, with the exception of myricetin derivatives (Table 2). The remaining variation included both intraspecific variation and measurement error.

3.2 | Relationships between chemical and nonchemical plant traits

3.2.1 | Incidence of polyphenols

Phylogenetic logistic regression was performed for 176 bivariate relationships. Of these, 89 were statistically significant ($p < .05$) or close-to-significant ($p > .05$ but $< .10$) when considering uncorrected *p*-values (Table S2; results summarised in Table 3). Twenty-eight of the relationships remained statistically significant at the $p < .05$ level following Benjamini–Hochberg correction (Table 3). The analyses suggested positive associations between seed mass, WSG, LMA and LamFT and the incidence of polyphenols, and negative associations between mean length of seed dormancy, RGR of saplings and trees, and physical seed defences and polyphenols. The relationship between tree height (H_{max}) and the incidence of polyphenols was typically positive, but a negative relationship was detected between H_{max} and the incidence of protein precipitation. Where statistically significant or close-to-significant coefficient values for growth form were detected they were sometimes positive and sometimes negative (Table 3 and Table S2).

3.2.2 | Polyphenol concentrations

Phylogenetic generalised least square regression was performed for 158 bivariate relationships. Sixteen were significant and another 14 close to statistically significant when considering uncorrected *p*-values. Seven were statistically significant following Benjamini-Hochberg correction of the *p*-values (Table 3 and Table S2). The results from

TABLE 1 Summary of the distribution of polyphenols and subgroups of polyphenols in seeds of 196 plant species on BCI

Type of compound	N	Prevalence (%)	Concentration		
			max	mean$_{all}$	mean$_{present}$
Tannin subgroups (TANN)	135	68.88	110.52	9.92	13.87
Hydrolysable tannins (HT)	70	35.71	89.69	3.87	9.60
Galloyl derivative (GALL)	63	32.14	29.86	1.32	3.81
HHDP derivative (HHDP)	39	19.90	63.23	2.55	11.11
Proanthocyanidins (PA)	120	61.22	63.82	6.05	10.02
Procyanidins (PC)	117	59.69	63.56	5.22	8.85
Prodelphinidins (PD)	56	28.57	24.31	0.83	2.84
Other polyphenol subgroups					
Flavonol glycosides (FLAV)	39	19.90	9.55	0.20	1.00
Kaempferol derivatives (KAEM)	26	13.27	6.45	0.13	1.12
Quercetin derivatives (QUER)	18	9.18	3.47	0.06	0.61
Myricetin derivatives (MYRI)	7	3.57	0.24	<0.01	0.17
Quinic acid derivatives (QUIN)	34	17.35	6.98	0.28	1.46
Total polyphenol subgroups (POLY)	148	75.51	110.82	10.41	13.62
Total phenolics (FC)	169	86.22	266.60	23.23	26.97

In the assessment of the prevalence of polyphenols (i.e. % of species in which each polyphenol group was detected), the full dataset was used (n = 566 samples representing 196 species, including samples that could not be fully dried). N refers to the number of species in which each compound group was detected. When summarising the maximum and mean concentrations of polyphenols (mg/g dry seed mass), only the subset of seed samples that could be fully dried was used. Mean concentrations are reported in two ways, either including (mean$_{all}$) or excluding (mean$_{present}$) species in which the compound group was not detected. The relationships between the different subgroups of polyphenols are outlined in Figure 1.

the PGLS analyses suggested positive associations (i.e. statistically significant or close-to-significant associations) between seed mass, WSG, LMA, LamFT and the concentrations of polyphenols, and negative associations between H_{max}, mean length of seed dormancy, RGR of saplings and trees, physical seed defences and the concentration of polyphenols. Where statistically significant or close-to-significant relationships were detected between quantitative chemical variables and nonchemical variables, the patterns were generally (in 15 out of 17 cases) in the same direction as any statistically significant or close-to-significant patterns detected in the corresponding analyses of incidence. In other words, nonchemical plant traits associated with the presence of polyphenols also tended to be associated with high concentrations of polyphenols in the subset of species in which they were present; Table 3 and Table S2).

The PCoA showed associations among RGR of saplings and trees, H_{max}, length of seed dormancy and seed physical defence vs. seed mass, adult physical defence (WSG, LamFT, LMA) and some of the polyphenols (Figure 2). The proportion of variance explained by

the two major PCoA axes was low (12.24% and 5.00% respectively). However, this is likely to be at least partially due to the large proportion of missing data for nonchemical variables in the data matrix. A parallel PCA analysis focusing on the most completely sampled traits yielded results that were qualitatively similar to those from the full PCoA and the proportion of variance explained rose to 32.55% and 16.01% for the first and second axes respectively (Appendix S3).

4 | DISCUSSION

While enemies killing seeds and young seedlings appear to play an important role in tropical forest communities (Bagchi et al., 2014; Harms et al., 2000; Mangan et al., 2010), surprisingly little is known about the defensive strategies of seeds and young seedlings (but see, e.g. Hanley & Lamont, 2002; Fricke & Wright, 2016). A particular gap in our knowledge is the lack of information about the expression of chemical defences at the early stages of the plant life cycle and

TABLE 2 Results from variance component analyses assessing the proportions of inter- vs. intraspecific variation in the concentrations of polyphenols

Trait	Interspecific (%)	Intraspecific + measurement error (%)
Tannin subgroups (TANN)	67.61	32.39
Hydrolysable tannins (HT)	79.42	20.58
Galloyl derivative (GALL)	80.95	19.05
HHDP derivative (HHDP)	82.07	17.93
Proanthocyanidins (PA)	71.52	28.48
Procyanidins (PC)	72.32	27.68
Prodelphinidins (PD)	70.66	29.34
Other polyphenol subgroups		
Flavonol glycosides (FLAV)	82.21	17.79
Kaempferol derivatives (KAEM)	79.73	20.27
Quercetin derivatives (QUER)	87.08	12.92
Myricetin derivatives (MYRI)	45.56	54.44
Quinic acid derivatives (QUIN)	81.21	18.79
Total polyphenol subgroups (POLY)	65.51	34.49
Total phenolics (FC)	66.68	33.31

the role of these defences in enhancing plant fitness. Our study on polyphenols in seeds in the woody plant community of BCI is to our knowledge the largest community-level study on seed polyphenols conducted to date, and indeed one of the largest community-level studies of polyphenols conducted at any single tropical forest site. We hope the broad taxonomic representation in our study provides a strong basis for future work to assess the generality of the patterns we observe at BCI across other tropical systems. The data reveal several interesting patterns:

Our data tentatively suggest that patterns of polyphenol production in seeds differ from those in the green parts of the plant. Although polyphenols appear to be nearly ubiquitous in seeds and many of the polyphenol groups targeted in our study were indeed detected in a substantial proportion of the studied plant species, the proportion of samples that did *not* contain polyphenols tended to be higher than in equivalent datasets on foliar chemistry. This was particularly noticeable for the three flavonol groups (myricetin, kaempferol and quercetin derivatives), which were detected in seeds of only 3.6%–13.3% of the plant species although they are one of the most common groups of polyphenols in green parts of plants (Salminen; unpublished data involving hundreds of different plant species). These observations suggest that the role of polyphenols might differ between seeds and leaves, for example, with some seed

polyphenols being produced primarily when physical seed defences are insufficient (see below). Nevertheless, until comparable datasets on seed and foliar chemistry have been collected for the same species, it will not be possible to draw any firm conclusions about differences in investment in polyphenol production between different life stages.

Overall, the phylogenetic logistic regression analyses, the PGLS analyses and the PCoA testing for associations between the incidence and concentration of polyphenols (or individual groups of polyphenols) and nonchemical plant traits yielded results that were qualitatively similar and in line with many of our predictions:

We predicted polyphenols to be positively associated with species-specific maximum tree height (prediction 1). In line with this prediction, the results from the bivariate analyses suggest that the likelihood of species possessing polyphenols in their seeds tends to increase with maximum tree height (H_{max}). However, for protein precipitation capacity and the concentration of kaempferol-based flavonols, we detected statistically significant relationships in the direction opposite to our prediction. The positive association between H_{max} and the incidence of several chemical compound groups are in line with our prediction that large trees might be more apparent to enemies than small trees (see, e.g. Schlinkert et al., 2015), leading to levels of polyphenol investment being associated with tree height. We note that such an increase in seed apparency with tree height could be relevant both in the context of pre- and post-dispersal seed enemies. Pre-dispersal enemies will likely encounter larger and more apparent seed crops on taller trees, but since H_{max} also tends to be positively associated with species-specific seed abundances at the community level (pers. obs.), the tallest tree species might also be the ones that are most apparent to post-dispersal enemies. Nevertheless, although the positive associations between polyphenol production and H_{max} are in line with predictions from plant apparency theory, we cannot rule out the possibility that these relationships are driven by selection for seed polyphenols in the context of some of their other functions, such as protecting the plant against harmful UV radiation (Waterman & Mole, 1994).

We predicted that the investment in seed polyphenols would be positively associated with the typical duration of seed dormancy (prediction 2). Contrary to this prediction, species with long seed dormancy times—and therefore more possibilities for accumulation of attack by post-dispersal enemies such as seed pathogens and vertebrate and invertebrate seed predators—appeared *less* likely to have polyphenols in their seeds than species with fast seed germination. The reason for this unexpected relationship remains open to speculation, but following Dalling, Davis, Schutte, and Arnold (2011) we suggest that the water impermeable endocarp that is often associated with species with long seed germination times might be sufficient to protect seeds from microbe attack, making investment in chemical defences less crucial. Our data did not allow us to assess whether species with different seed dormancy types (e.g. species possessing physical dormancy vs. other species) differ in their polyphenol profiles. Future studies specifically designed to target this question have the potential to yield novel insights into the ecological roles of seed polyphenols.

TABLE 3 Summary of results from analyses testing for associations between selected nonchemical traits and the incidence (phylogenetic logistic regressions) and concentration (PGLS regressions) of the chemical groups [Colour table can be viewed at wileyonlinelibrary.com]

	H_{max} (1)	log(Mean length of dorm.) (2)	log($RGR_{saplings}$) (3)	log(RGR_{trees}) (3)	log(Seed mass) (4)	Seed protection (5)	Relative toughness (5)	Relative peak force (5)	WSG (6)	LMA (6)	LamFT (6)	Growth form (7)

Predictions

Phylogenetic logistic regressions

FC, POLY, TANN, HT, GALL, HHDP, FLAV, MYRI, KAEM, QUER, PA, PC, PD, QUIN, PPC, PRO-OX

PGLS regressions

FC, POLY, TANN, HT, GALL, HHDP, FLAV, MYRI, KAEM, QUER

(Continues)

TABLE 3 (Continued)

	H_{max} (1)	log(Mean length of dorm.) (2)	log($RGR_{saplings}$) (3)	log(RGR_{trees}) (3)	log(Seed mass) (4)	Seed protection (5)	Relative toughness (5)	Relative peak force (5)	WSG (6)	LMA (6)	LamFT (6)	Growth form (7)
PA												
PC												
PD												
QUIN				NA			NA	NA				
PPC							NA	NA		*		
PRO-OX												

Shown are also results from analyses assessing the relationship between the nonchemical variables and protein precipitation (PPC) and oxidative capacity (PRO-OX). Statistically significant or close-to-significant positive and negative associations are shown in red and blue, respectively. The darkest shading indicates statistically significant associations ($p < .05$) following Benjamini-Hochberg correction of p-values, the intermediate shading shows associations that are statistically significant ($p < .05$) if considering raw p-values (i.e. no correction for multiple testing), and the light shading shows associations that are marginally nonsignificant (raw $p > .05$ but $<.10$). In the analyses of the effect of growth form on the incidence and concentrations of polyphenols, lianas were set as the reference group (intercept). Hence, a red colour indicates that the polyphenol group in question is more likely to occur or, where present, occurs in higher concentrations in trees than in lianas, whereas a blue colour indicates the opposite. 'NA' indicate relationships between chemical and nonchemical variables that were not assessed due to small sample sizes (or in the case of the logistic regressions low prevalence of the compound group in the data set) while asterisks denote models that did not converge. The numbers in brackets associated with the nonchemical traits refer to the seven predictions outlined in the introduction. Abbreviations of nonchemical variables are as follows: H_{max}, maximum tree height; $RGR_{saplings}$, relative growth rate of saplings; RGR_{trees}, relative growth rate of trees; WSG, wood-specific gravity; LMA, leaf mass per area; LamFT, lamina fracture toughness; PGLS, phylogenetic generalised least square. See Figure 1 for a definition of the abbreviations of chemical variables.

Our data revealed several negative associations between seed polyphenols (condensed tannins in particular) and the relative growth rates of saplings and adult trees. This provides support for prediction 3, that is, a negative relationship between investment in seed polyphenols and relative growth rates. A recent meta-analysis by Endara and Coley (2011) focusing on seedlings, saplings and adult plants found strong support for the key predictions of the resource availability hypothesis, including a negative relationship between investment in defences and relative growth rates. Our study adds new insights by showing that the kind of growth-defence trade-offs commonly documented within particular ontogenetic stages can also occur between different stages of the plant life cycle. In other words, functional traits of larger plants are reflected in the chemical contents of their seeds.

A key aim of our study was to assess whether seed polyphenols are associated with nonchemical seed traits and defence traits expressed at later life stages. In line with prediction 4, that is, a positive relationship between seed mass and investment in polyphenols, polyphenol content was often positively associated with seed mass. This suggests that species with few opportunities for reproduction (because the number of offspring produced is negatively correlated with seed mass; Muller-Landau, Wright, Calderón, Condit, & Hubbell, 2008) are indeed the ones to invest most in chemical seed defences.

We hypothesised that investment in multiple types of seed defences might be costly, and therefore predicted a negative association between investment in mechanical and chemical seed defences (prediction 5). The observed negative relationship between the investment in physical seed protection and the incidence—and in some cases concentrations—of polyphenols suggests that there might be trade-offs between investment in mechanical and chemical seed defences. When faced with limited resources, the most cost-effective strategy for plants might be to invest in one key mode of seed defence rather than to deploy multiple types of defences. The negative associations between seed polyphenol content and physical seed protection is analogous to patterns documented in leaf defences, where trade-offs between physical and chemical defences have been documented at both inter- and intraspecific levels (e.g. Hanley & Lamont, 2002; but see, for example, Koricheva et al., 2004). Given the negative association between seed mass and investment in physical seed protection (Table S1), care should be taken in drawing too strong conclusions about the main driver of this potential trade-off. Nevertheless, relative toughness and relative peak force are seed mass-corrected variables, suggesting that the statistically significant relationships between polyphenol content and investment in physical seed protection (variable 'Seed protection' in Table 3) are not simply caused by a correlation between polyphenol content and seed mass.

While there appears to be a negative trade-off between chemical and physical defences at the seed level, our study revealed many significant *positive* associations between investment in seed polyphenols and investment in nonchemical plant defence traits expressed at later stages of the plant life cycle, in line with prediction 6. In fact, some of the clearest patterns to emerge between polyphenol content and nonchemical plant traits were the positive associations between polyphenols and WSG, LMA, and LamFT. Given that plant
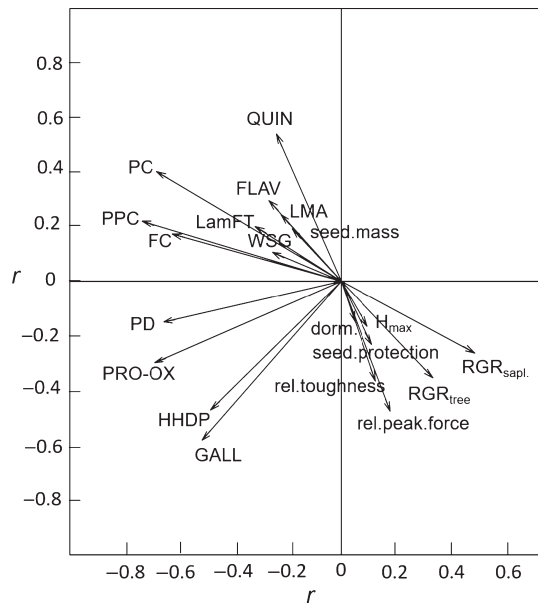
FIGURE 2 Relationships between individual chemical and nonchemical traits and the first two PCoA axes (PCoA1 and PCoA2). The correlation between each trait and the two PCoA axes was assessed using Pearson's correlation coefficient (r; Table S3). The length of the vectors are proportional to the sizes of the correlation coefficients. Abbreviations of chemical variables are given in Figure 1. Abbreviations of nonchemical variables are as follows: dorm., mean length of dormancy; H_{max}, maximum tree height; LamFT, lamina fracture toughness; LMA, leaf mass per area; rel.peak.force, relative peak force; rel.toughness, relative toughness; RGR_{sapl}, relative growth rate of saplings; RGR_{tree}, relative growth rate of trees; WSG, wood-specific gravity

defences can vary considerably across plant ontogeny (e.g. Barton & Boege, 2017), this is an interesting observation. The relatively strong associations between seed polyphenols and traits expressed at later life stages provide a potential explanation for the commonly documented correlations between seed mass and the growth and survival of trees in larger size classes (e.g. Visser et al., 2016; Wright et al., 2010): species tend to show consistent patterns in their investment in defences throughout their life cycle. Our data also suggest that the suite of correlated functional plant traits that are associated with regeneration in high-light forest gaps vs. in the shaded understory (such as seed mass, relative growth rates and structural leaf defences; e.g. Coley, 1983; Ghazoul & Sheil, 2010) also appears to be manifested in the polyphenol content of seeds, with potential implications for community-level patterns of seed and seedling performance.

Based on differences in life history strategies between trees and lianas (Asner & Martin, 2012), we predicted that polyphenol investment would be higher in trees than in lianas (prediction 7). Where statistically significant or close-to-significant coefficient values for growth form were detected in the bivariate analyses they were sometimes positive and sometimes negative, suggesting that there are no consistent differences between trees and lianas in their investment in seed polyphenols and that other factors than growth form are likely

to be more important determinants of the seed polyphenol profile of woody plants.

Of the seven predictions evaluated in this study, two (predictions 1–2) were motivated by plant apparency theory and five (predictions 3–7) by theories based on resource availability and life history consideration. While our data provide mixed support for plant apparency being an important driver of the macroevolution of seed polyphenols, the documented community-level patterns of polyphenol distribution are consistent with predictions from the resource availability hypothesis (Coley et al., 1985) and suggest that the growth environment of a species is an important predictor of the degree of investment in chemical seed defences. Our study thus adds to recent studies that have found limited support for plant apparency theory or have found resource availability to be a better predictor of investment in plant defences than plant apparency (Endara & Coley, 2011; Smilanich et al., 2016). Nevertheless, since plant apparency can be assessed in a large number of ways of which our study only assessed two, caution needs to be taken before concluding that apparency is consistently an unimportant driver of the evolution of seed defences.

In summary, our study shows that the distribution of polyphenols in seeds across species in the diverse plant community of BCI is nonrandom and can be explained by a number of commonly studied functional plant and seed traits. The observation that chemical defence expression in seeds appears to be negatively associated with physical seed defence and positively associated with physical defence of conspecific leaves (LamFT and LMA) and stems (wood density) has to our knowledge not been documented before. While much is still to be learnt about the roles of different types of seed polyphenols in contemporary ecological processes, the patterns revealed by our study provide a potential answer to the outstanding question of why seed traits such as size continue to have strong correlations with performance of adult plants decades to centuries after the seed is long gone.

AUTHOR CONTRIBUTIONS

S.G., J.R. and J.-P.S. conceived the idea for the study; S.G., P.-C.Z. and S.J.W. coordinated the collection of samples to be used for the

chemical analyses which were conducted by J.K. and J.-P.S.; S.J.W., N.G. and E.F. collected data on nonchemical traits; Data analyses were conducted by S.G. and J.R., with substantial inputs from E.F. and S.J.W.; S.G. led the writing of the manuscript with contributions from all authors. All authors have given their final approval for publication.

DATA ACCESSIBILITY

Data on chemical and nonchemical traits are available from the Dryad Digital Repository https://doi.org/10.5061/dryad.5r083 (Gripenberg et al., 2017).

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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