Biotic and abiotic plant-soil feedback depends on nitrogen-acquisition strategy and shifts during long-term ecosystem development

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Abstract

1. Feedback between plants and soil is an important driver of plant community structure, but it remains unclear whether plant-soil feedback (PSF): (i) reflects changes in biotic or abiotic properties, (ii) depends on environmental context in terms of soil nutrient availability, and (iii) varies among plant functional groups. Because soil nutrient availability strongly affects plant distribution and performance, soil chemical properties and plant nutrient-acquisition strategies might serve as important drivers of PSF.

2. We used soils from young and old stages of a long-term soil chronosequence to represent sites where productivity is limited by nitrogen (N) and phosphorus (P) availability, respectively. We grew three N-fixing and three non-N-fixing plant species in soils conditioned by co-occurring conspecific or heterospecific species from each of these two stages. In addition, three soil treatments were used to distinguish biotic and abiotic effects on plant performance, allowing measurements of overall, biotic and abiotic PSF.

3. In young, N-poor soils, non-N-fixing plants grew better in soils from N-fixing plants than in their own soils (i.e. negative PSF). However, this difference was not only associated with improved abiotic conditions in soils from N-fixing plants, but also with changes in soil biota.

4. By contrast, no significant PSF was observed for N-fixing plants grown in young soils. Moreover, we did not observe any significant PSF for either N-fixing or non-N-fixing plants growing in old, P-impoverished soils.

5. *Synthesis.* The direction and strength of PSF varied among N-acquisition strategies and soils differing in nutrient availability, with stronger PSF in younger, N-poor soils compared to older, P-impoverished soils. Our results highlight the importance of considering soil nutrient availability, plant-mediated abiotic and biotic soil properties, and plant nutrient-acquisition strategies when studying PSF, thereby advancing our mechanistic understanding of PSF during long-term ecosystem development.

KEYWORDS

chronosequence, Fabaceae, long-term ecosystem development, nutrient-acquisition strategies, plant-soil feedback, plant-soil (below-ground) interactions, plant functional traits, ecosystem retrogression

1 | INTRODUCTION

Plants modify both abiotic and biotic soil properties that consequently influence the performance of other conspecific or heterospecific plants, a mechanism known as 'plant-soil feedback' (PSF) (Bever, Westover, & Antonovics, 1997; Ehrenfeld, Ravit, & Elgersma, 2005; van der Putten et al., 2013). Negative PSF is typically associated with the accumulation of host-specific soil-borne pathogens (Bever, 2003; Klironomos, 2002; Mangan et al., 2010), and can promote species coexistence and diversity within plant communities (e.g., Bever, 2003; Bever et al., 1997; Bonanomi, Giannino, & Mazzoleni, 2005). However, negative PSF driven by pathogens might be more strongly expressed in short-term studies where soil biota are conditioned experimentally; conversely, longer-term effects of abiotic soil conditioning via leaf and root litter and root activity have received less attention (Bardgett & Wardle, 2010; Berendse, 1998). In addition, environmental conditions such as soil nutrient availability strongly affect plant performance and influence abiotic and biotic feedback processes within plant communities. However, few studies have considered the environmental context-dependency of PSF over long ecological time-scales (de Kroon et al., 2012; Kardol, de Deyn, Laliberté, Mariotte, & Hawkes, 2013; Revillini, Gehring, & Johnson, 2016; Reynolds, Packer, Bever, & Clay, 2003). Determining the relative importance of the longer-term abiotic and shorter-term biotic PSF processes and their dependence on environmental context could help us better understand plant species distributions across ecosystems and plant community responses to global change (Bardgett & Wardle, 2010; Lekberg et al., 2018; Smith-Ramesh & Reynolds, 2017; van der Putten, Bradford, Brinkman, van de Voorde, & Veen, 2016).

Environmental conditions are expected to influence the strength and direction of biotic and abiotic PSF (e.g., de Kroon et al., 2012; Kardol et al., 2013; Manning, Morrison, Bonkowski, &

Bardgett, 2008; Revillini et al., 2016; Reynolds et al., 2003). In particular, major changes in soil nitrogen (N) and phosphorus (P) availability during long-term ecosystem development strongly affect plant performance (Coomes, Bentley, Tanentzap, & Burrows, 2013; Laliberté et al., 2012; Vitousek, 2004), and could promote certain abiotic and/or biotic feedback associated with a particular plant nutrient-acquisition strategy (Baxendale, Orwin, Poly, Pommier, & Bardgett, 2014; Lekberg et al., 2018; Revillini et al., 2016). For example, low soil N availability in the early stages of ecosystem development limits plant productivity (Laliberté et al., 2012; Vitousek & Farrington, 1997; Walker & del Moral, 2003), but favours N-fixing plants that form mutualistic associations with N-fixing microorganisms to acquire atmospheric N (Vitousek, Menge, Reed, & Cleveland, 2013; Walker, 1993). Consequently, N-fixing plants are expected to generate positive effects on plant growth via abiotic PSF in young soils, by increasing soil N availability (Bellingham, Walker, & Wardle, 2001; Vitousek & Walker, 1989).

As soil P availability decreases with soil development due to prolonged erosion and occlusion (Turner & Laliberté, 2015; Vitousek, 2004; Walker & Syers, 1976), N-fixing plants are expected to become less favoured due to the high P costs associated with symbiotic N fixation (Hartwig, 1998; Raven, 2012). Conversely, a greater diversity of plant species possessing a variety of P-acquisition strategies is increasingly favoured as soil P availability declines (Lambers, Raven, Shaver, & Smith, 2008; Zemunik, Turner, Lambers, & Laliberté, 2015), some of which depend on specific groups of soil biota (e.g., mycorrhizal fungi; Smith, Anderson, & Smith, 2015). As such, biotic PSF mechanisms might become more prominent on weathered, severely P-impoverished soils for two reasons. First, an increasingly efficient P-acquisition strategy based on ephemeral roots with biochemical adaptations (e.g., high specific root length, root hairs, carboxylate release) could trade off against pathogen defence (Albornoz, Burgess, Lambers, Etchells, & Laliberté, 2016; Lambers et al., 2018; Newsham, Fitter, & Watkinson, 1995), potentially leading to strong pathogen-mediated negative density dependence (Laliberté, Lambers, Burgess, & Wright, 2015). On the other hand, interactions with mutualistic soil microbes, such as arbuscular mycorrhizal (AM) and ectomycorrhizal

(ECM) root associations or plant growth-promoting rhizobacteria, may offer protection or enhance defence against pathogens (Albornoz et al., 2016; Azcón-Aguilar & Barea, 1997; Bennett et al., 2017; Laliberté et al., 2015; Lambers et al., 2018; Smith & Read, 2008). However, it remains unclear how the net effect of pathogens and mutualists affect N-fixing and non-N-fixing plants in a manner that influences PSF interactions in weathered, severely P-impoverished soils.

Well-defined, long-term soil chronosequences forming natural nutrient-availability gradients provide useful model systems to explore how abiotic and biotic PSF mechanisms of plant communities respond to shifts in environmental context over a longer time-scale (Kardol et al., 2013; Laliberté et al., 2013; Lekberg et al., 2018; Walker, Wardle, Bardgett, & Clarkson, 2010). In this study, the environmental context of interest is soil nutrient availability, particularly of N and P. The declining soil fertility and contrasting shifts in soil nutrient limitation of plant growth - from N to P limitation with soil age (Laliberté et al., 2012; Vitousek & Farrington, 1997; Walker & Syers, 1976) - along long-term soil chronosequences influence the relative abundance of N-fixing and non-N-fixing plant species (Chapin, Walker, Fastie, & Sharman, 1994; Vitousek & Walker, 1989; Walker, 1993; Zemunik, Turner, Lambers, & Laliberté, 2016). However, whether these shifts in vegetation are associated with changes in underlying abiotic and biotic PSF mechanisms remains to be determined.

To examine how abiotic and biotic PSF interactions among naturally co-occurring plant functional groups change with shifts in soil nutrient availability during long-term ecosystem development, we grew N-fixing and co-occurring non-N-fixing plant species in a glasshouse in sterilised and unsterilised soils. These soils were field-conditioned by the respective mature plants found on two contrasting dune systems representing >120,000 years of ecosystem development along the Jurien Bay coastal dune chronosequence (Laliberté et al., 2012; Laliberté, Zemunik, & Turner, 2014; Turner & Laliberté, 2015). This chronosequence forms a strong natural gradient in soil nutrient availability, particularly with regard to P and N (Laliberté et al., 2012; Turner & Laliberté, 2015). We hypothesised that:

(H1) Abiotic PSF is more important in young, N-poor soils than in old, severely P-impoverished soils, because N-fixing plants increase soil N availability (via N fixation) in young soils, while biotic PSF is predominant in old soils. Biotic PSF is likely important in old soils due to many plants possessing root traits that enhance P acquisition at the expense of defence against soil-borne pathogens, and root traits enabling mutualistic associations with soil microbes that assist with nutrient acquisition and/or pathogen defence (Laliberté et al., 2015; Lambers et al., 2018).

(H2) The magnitude and direction of PSF in young soils differ between N-fixing and non-N-fixing plants. Specifically, we expected that non-N-fixing plants would grow better in soils conditioned by N-fixing plants (through having higher N availability) than in soils conditioned by non-N-fixing plants, while growth of N-fixing plants would be less in soils from non-N-fixing plants and would be unresponsive to soils from heterospecific N-fixing plants relative to conspecifics.

(H3) Shifts in PSF for both N-fixing and non-N-fixing plants depend on soil age. In particular, we expected that any positive PSF detected in young, N-poor soils would become increasingly negative in old, severely P-impoverished soils, and would be of similar magnitude for plants in old soils from both N-fixing and non-N-fixing plants.

Addressing these hypotheses in combination will help determine how the relative importance of biotic and abiotic PSF among N-fixing and non-N-fixing plant functional groups depends on shifts in soil nutrient availability. This will advance our understanding of the roles of PSF in the interactions among plant functional groups during long-term ecosystem development.

2 | MATERIALS AND METHODS

2.1 | Experimental design

A glasshouse experiment was set up using field-collected soils, with the following treatment combinations: two dune systems (young, old) × six soil origins (collected from beneath three N-fixing and three non-N-fixing species, representing two plant functional groups; Table 1) × three soil

145 treatments [‘overall’ soil (unsterilised soil collected beneath plants belonging to each of the studied
146 plant species), ‘abiotic’ soil (soil collected beneath each studied plant species and sterilised), and
147 ‘biotic’ soil (sterilised bulk soil inoculated with soil collected beneath each studied plant species)] ×
148 six plant species (three N-fixing and three non-N-fixing species; Table 1). Soils were collected across
149 three plots (10 m × 10 m) within each dune system, and the experimental design was treated as a
150 randomised block design with the individual plots treated as ‘blocks’. Each treatment combination
151 was replicated six times, resulting in a total of 1,296 individual pots, each containing a single plant.

152 **2.2 | Study area**

153 Collection of ‘field-conditioned’ soils was done in two contrasting dune systems representing
154 >120,000 years of soil and ecosystem development along the Jurien Bay dune chronosequence
155 (30°01' to 30°24' S; 114°58' to 115°11' E; Hayes, Turner, Lambers, & Laliberté, 2014; Laliberté et al.,
156 2012; Turner & Laliberté, 2015), which is located in the southwest Australian biodiversity hotspot
157 (Hopper & Gioia, 2004). The Quindalup Young dune system [<100 years old; corresponding to stage
158 1 in Hayes et al. (2014)] has highly calcareous soils displaying limited or no soil development and
159 showing high P availability (Turner & Laliberté, 2015), but low N availability that limits plant
160 productivity (Hayes et al., 2014; Laliberté et al., 2012). By contrast, the older Spearwood dune
161 system [>120,000 years old; corresponding to stage 4 in Hayes et al. (2014)] shows weathered,
162 nutrient-depleted soils, in which low P availability limits plant productivity (Hayes et al., 2014;
163 Laliberté et al., 2012). Soils from both selected dune systems are derived from the same parent
164 material (calcareous sand of a marine origin; Turner & Laliberté, 2015) and share the same regional
165 flora (Laliberté et al., 2014). Other soil properties, vegetation and climate of the dune systems and
166 corresponding study plots have been described in detail elsewhere (Laliberté et al., 2012; Turner,
167 Hayes, & Laliberté, 2018; Turner & Laliberté, 2015; Zemunik et al., 2015, 2016).

168 **2.3 | Plant species selection**

169 Three common plant species from the N-fixing and non-N-fixing plant functional groups of each
170 dune system were identified using vegetation survey data described by Zemunik et al. (2015, 2016;
171 Table 1). For each of the two dune systems, the three species from each of these two N-acquisition
172 strategies were also selected based on the following two criteria: (i) the species grows as a tree or
173 large shrub at maturity, and (ii) its seeds lack complex endogenous dormancy mechanisms. In
174 addition, the non-N-fixing species were selected to represent the range of belowground nutrient-
175 acquisition strategies found on the respective dune system (Zemunik et al., 2015; Table 1).

176 **2.4 | Soil collection, sterilisation and potting**

177 Soils were collected beneath each of the selected plant species from three plots (10 m × 10 m) for
178 each of the two dune systems (Table 1). Soils from the top 20 cm layer were collected beneath a
179 total of 60 large individuals of each plant species (<1 m from base of stem) across three plots, using
180 75-mm diameter sand augers. Bulk soil (0 - 20 cm depth) from each plot was also collected at every
181 metre mark, where plants and/or plant canopies were absent, along three randomly selected 10-m
182 line transects. Soil samples were collected over four days in June 2014, when the study area
183 experiences relatively high rainfall and the soil microbial community is expected to be most active in
184 the surface soil layers in the seasonally dry Mediterranean climate that characterises the area (Teste
185 et al., 2016).

186 Field-collected soils for each soil origin (i.e. from beneath each plant species) and bulk soil
187 from each respective plot were separately homogenised with a cement mixer before packaging in
188 separate polyethylene bags. For logistical reasons, it was necessary to homogenise bulk soil or soil by
189 species origin within each individual plot; however, this method of soil mixing maintains each plot
190 within each dune system as an independent spatial replicate. Soils required for the overall (i.e. field-
191 untreated/unsterilised) soil treatment and for the preparation (i.e. inoculation) of the biotic soil
192 treatment were untreated and stored in a cool (~17 °C), dark room prior to potting. Bulk soils
193 (required for the preparation of biotic soil treatment) and soils of different species origins required

for the abiotic soil treatment were sterilised with gamma (γ)-irradiation (50 kGy; Steritech NSW, Australia). Gamma-irradiation is a method of sterilisation that effectively sterilises soils, while exerting minimal impact on other soil properties (Berns et al., 2008). Each species 'soil origin' × 'biotic soil treatment' combination was prepared by inoculating γ-sterilised bulk soil with 7% (v/v) untreated soil collected beneath the respective plant species from the same plot (i.e. 'block'). The use of γ-sterilised bulk soils enabled testing of biotic plant-soil conditioning effects by minimising the variability of chemical properties among soils collected beneath co-occurring plant species (van de Voorde, van der Putten, & Bezemer, 2012). Each soil treatment-combination was potted into 1.2 L free-draining pots, filled with 1.1 L of soil per pot.

Subsamples of field-collected soils of different species origins and the corresponding γ-irradiated soils from each plot were analysed for relevant soil chemical properties (see Table S1 in Supporting Information). All soil-collection and potting equipment was cleaned and then sterilised with 70% (v/v) ethanol between soil treatment-combination types to avoid cross-contamination.

2.5 | Glasshouse experiment setup and harvest

Seeds were bought from Nindethana Seed Service (Albany, Australia) and were collected from provenances as close as possible to the study area (Table S2). Seeds of species possessing physical or physiological dormancy were pre-treated according to Sweedman & Merritt (2006). Following pre-treatment, seeds were surface-sterilised with a 2% (w/v) sodium hypochlorite solution for 30 min before rinsing three times with sterile deionised water and incubated (~17 - 25 °C) under sterile conditions using 0.5% (w/v) water-agar (Sigma-Aldrich, Castle Hill, Australia) in Petri dishes. Due to seed-viability issues, instead of seeds, *Olearia axillaris* softwood stem cuttings (~5 cm long and as uniform as possible; Table S2) were used, and propagated on triple-pasteurised, nutrient-poor soil for a period of six weeks by Australian Native Nurseries (Oakford, Australia) prior to transplanting.

For each species except *Olearia axillaris*, three young seedlings were transplanted into each designated treatment pot within two days following observation of radicle emergence. Initial fresh biomass of each *Olearia axillaris* cutting was weighed, surface-sterilised with 2% (w/v) sodium

hypochlorite solution for 5 min, and then rinsed three times with sterile deionised water; following this, two cuttings were transplanted into each designated treatment pot. Plants of each species were thinned to a single, similar-sized seedling or cutting per pot one week after transplanting.

Plants were grown in a glasshouse and drip-irrigated to maintain approximately 70% field soil moisture capacity. Each plant species was grown for six months before whole plants were harvested. Plant samples were dried at 60 °C for 72 h before total plant dry biomass was measured. Plant growth of *Olearia axillaris* cuttings was determined by the difference between initial and final plant biomass of the cuttings.

2.6 | Soil chemical analyses

Three to four subsamples of untreated and γ-sterilised soils of each plant species origin were established from soils collected across three plots within either the young or the old dune system. Untreated and γ-sterilised soils were passed through a 2-mm sieve before soil chemical properties were analysed. Measurements of total P, nitrate (NO_3^-) and ammonium (NH_4^+) concentrations were taken on untreated field soil subsamples only, while measurements of soil pH, total carbon, total N, readily exchangeable phosphate (resin P), exchangeable cation-exchange capacity, exchangeable potassium and exchangeable sodium concentrations were taken on both untreated and γ-sterilised soil subsamples. The soil chemical measurements that we performed for the γ-sterilised soil subsamples are those that are known to be potentially most strongly modified by the sterilisation treatment (Berns et al., 2008; McNamara, Black, Beresford, & Parekh, 2003). Soil [NO_3^-] and [NH_4^+] were determined using a KCl extraction method (Gianello & Bremner, 1986; Øien & Selmer-Olsen, 1980) on a Technicon AutoAnalyzer (Technicon Instruments, NY, USA). Methods for measuring other soil chemical parameters have been described elsewhere (Laliberté et al., 2012; Turner & Laliberté, 2015).

2.7 | Statistical analyses

Log-response ratios (LRR) were used to calculate overall, biotic or abiotic effect sizes of plant-soil feedback (Brinkman, van der Putten, Bakker, & Verhoeven, 2010). Within each dune system of contrasting soil age (young, old) and soil treatment type (overall, biotic, abiotic), the feedback response of each plant grown in soils conditioned by co-occurring heterospecific plants in general (i.e. all heterospecific species), N-fixing, or non-N-fixing plant species was calculated as $\log_{10}(B_C/B_{AH})$, $\log_{10}(B_C/B_{AHNF})$, or $\log_{10}(B_C/B_{AHX})$, respectively; where B_C is the plant biomass of an individual replicate grown in soil of conspecific origin, B_{AH} is the average plant biomass in soils of all co-occurring heterospecific species origins from the same given plot, B_{AHNF} is the average plant biomass in soils of all co-occurring N-fixing heterospecific species origins from the same given plot, and B_{AHX} is the average plant biomass in soils of all co-occurring non-N-fixing heterospecific species origins from the same given plot. All feedback values in this study were calculated as a relative response of growth in soils conditioned by conspecifics versus that in a given soil conditioned by heterospecific plants. As such, to maintain brevity, PSF will hereafter also be expressed as feedback in a given soil conditioned by heterospecific plants.

The common logarithm [$\log_{10}(x)$] was used to calculate LRR in this study for ease of interpretation: a single unit increase indicates that plant growth in conspecific soil origin is ten times greater than the average plant growth in soils from co-occurring heterospecific N-fixing or non-N-fixing or all (i.e. N-fixing and non-N-fixing) species origins, while a zero LRR value indicates no feedback effect. In addition, we selected LRR to calculate feedback effect sizes for the following reasons: (1) it allows simple relative comparisons of plant performance among plant functional groups containing multiple plant species with different growth rates/forms, and between dune systems of contrasting soil ages (Brinkman et al., 2010); (2) the average effect of soil conditioning by a plant functional group, rather than species-level pair-wise feedback interactions (e.g., 'interaction coefficient' calculation method; Bever et al., 1997; Kulmatiski & Kardol, 2008), is the primary factor of interest in this study; and, (3) the logarithmic transformation of biomass-ratio values enables the

calculation of feedback values that are proportional to the original effect sizes which, in turn, allows accurate comparisons among feedback effects that work in different directions (Brinkman et al., 2010).

Linear mixed-effects (LME) models (Zuur, Ieno, Walker, Saveliev, & Smith, 2009) were used to compare the differences in PSF responses among plant functional groups (N-fixing, non-N-fixing), plant functional group soil origins (from heterospecific N-fixing, non-N-fixing, or all plant functional groups), dune systems of contrasting soil ages (young, old), and the interaction among these three factors within each soil treatment (overall, biotic, abiotic). Differences among feedback responses of plant functional groups, plant functional group soil origins, and soil ages were of primary interest, with blocking (by plot) and species soil origin (i.e. soil collected from beneath a specific plant species) treated as random factors in the models. The significance of the direction (positive or negative) of each feedback value was determined by fitting the respective model without intercept terms. The following combination of results from the analyses of overall, biotic and abiotic feedback would support our specific hypotheses:

(H1) Our first hypothesis that abiotic PSF is more important in young soils than in old soils where biotic PSF is predominant, would be supported by non-zero abiotic PSF in the young soils and non-zero biotic PSF in the old soils, which operate in the same direction as the corresponding non-zero overall PSF. In addition, biotic PSF in the young soils and abiotic PSF in the old soils would be expected to be either dissimilar in direction to the corresponding overall PSF or neutral (i.e. no PSF).

(H2) Our second hypothesis that differences in the direction of overall (and abiotic) feedback between plant functional groups in young soils depend on plant functional type (N-fixing and non-N-fixing), would be supported by negative PSF of non-N-fixing plants and neutral PSF of N-fixing plants grown in young soils conditioned by heterospecific N-fixing plants (compared with soils from conspecifics). It would also be supported by neutral PSF of non-N-fixing plants and

positive PSF of N-fixing plants grown in young soils conditioned by heterospecific non-N-fixing plants.

(H3) Our third hypothesis that shifts in the direction and magnitude of PSF depend on soil age, would be supported by changes in the direction of any non-negative overall PSF detected in young, N-poor soils to negative overall (and biotic) PSF of similar magnitude in old, P-impoverished soils, for both N-fixing and non-N-fixing plant functional groups.

All soil chemical properties were compared using LME models among untreated soils that were field-conditioned by N-fixing and non-N-fixing plant functional groups, dune systems of contrasting soil ages, the corresponding γ-irradiated treatments (if applicable), and the interaction among these factors, with blocking and plant species soil origin treated as random factors.

For all LME models, standardised residuals were visually inspected to verify model assumptions. Akaike Information Criterion and likelihood-ratio tests were used to identify appropriate models (Zuur et al., 2009), and *post hoc* Tukey tests were subsequently performed when a main or interaction term was significant (Hothorn, Bretz, & Westfall, 2008). Statistical analyses were performed in 'R' (R Core Team, 2015) using 'nlme' (Pinheiro, Bates, DebRoy, Sarkar, & R Core Team, 2014) and 'multcomp' (Hothorn et al., 2008) packages.

3 | RESULTS

3.1 | H1: Relative importance of biotic and abiotic plant-soil feedback

In the young, N-poor dune system, non-N-fixing plants grew better in soils from all co-occurring heterospecific plants compared with those in soils from conspecifics (i.e. negative PSF; Figure 1; Tables S3, S4 and S5). The negative overall PSF detected in non-N-fixing plants from the young dune system was primarily driven by their positive response to a combination of biotic and abiotic soil conditioning by co-occurring N-fixing plants (Figure 1; Tables S3, S4 and S5). Conversely, all plants from the old, severely P-impoverished dune system, together with N-fixing plants from the young dune system, generally showed similar growth in soils conditioned by all co-occurring heterospecific

plants compared with those in soils from conspecifics (i.e. neutral PSF; Figure 1; Tables S3, S4 and S5).

For both the overall (Figure 1a; Table S3) and abiotic (Figure 1c; Table S5) soil treatments in the young dune system, the mean feedback values of non-N-fixing plants in soils conditioned by N-fixing plants were significantly less than that for non-N-fixing plants in soils conditioned by heterospecific non-N-fixing plants. However, within the biotic soil treatment in the young dune system, the mean feedback value of non-N-fixing plants in soils conditioned by N-fixing plants was similar to that for non-N-fixing plants in soils conditioned by heterospecific non-N-fixing plants (Figure 1b; Table S4).

The significantly greater growth of non-N-fixing plants from the young, N-poor dune system in sterilised soils collected beneath co-occurring heterospecific N-fixing plants compared with that in sterilised soils collected beneath conspecifics (i.e. negative abiotic PSF) might be associated with greater [NH_4^+] measured in young soils beneath N-fixing plants (Figure 2; Table S1). This is because no other measured soil chemical properties differed significantly between soils collected beneath non-N-fixing and N-fixing plants in the young dune system (Table S1).

3.2 | H2: Plant-soil feedback among nitrogen-fixing plants and non-nitrogen-fixing plants

In young, N-poor soils conditioned by either all co-occurring heterospecific plants or by heterospecific N-fixing plants, growth responses among non-N-fixing plants and N-fixing plants were different (Figure 1; Tables S3, S4 and S5). That is, within each soil treatment (i.e. overall, biotic and abiotic), non-N-fixing plants produced less biomass in young soils conditioned by conspecifics compared with those in soils from either all co-occurring heterospecific plants or co-occurring N-fixing plants (i.e. negative PSF; Figure 1; Tables S3, S4 and S5). In contrast, the growth of N-fixing plants was similar in young soils from conspecifics and in soils from all co-occurring heterospecific plants (i.e. neutral PSF; Figure 1; Tables S3, S4 and S5). However, mean feedback values of N-fixing and non-N-fixing plants grown in young soils conditioned by all co-occurring heterospecific plants or

by co-occurring N-fixing plants were similar within each soil treatment (Figure 1; Tables S3, S4 and S5).

3.3 | H3: Changes in plant-soil feedback as dependent on soil age

The strength and direction of PSF experienced by non-N-fixing plants, but not N-fixing plants, depended on soil age (Figure 1; Tables S3, S4 and S5). That is, across all soil treatments (i.e. overall, biotic and abiotic), non-N-fixing plants produced less biomass in young, N-poor soils conditioned by conspecifics compared with those in soils from either all co-occurring heterospecific plants or co-occurring N-fixing plants (i.e. negative PSF; Figure 1; Tables S3, S4 and S5). By contrast, non-N-fixing plants showed similar growth responses in old, severely P-impoverished soils conditioned by conspecifics and in soils from all co-occurring heterospecific plants (i.e. neutral PSF; Figure 1; Tables S3, S4 and S5). However, N-fixing plants did not show any significant PSF response for either of the dune systems (Figure 1; Tables S3, S4 and S5). The mean feedback values of non-N-fixing plants from the young and old dune systems in soils conditioned by all co-occurring heterospecific plants or by co-occurring N-fixing plants were similar within each soil treatment type (Figure 1; Tables S3, S4 and S5).

4 | DISCUSSION

Our results show that PSF during long-term ecosystem development depends on soil nutrient availability and plant N-acquisition strategy. In young soils, where productivity is limited by N availability (Hayes et al., 2014; Laliberté et al., 2012; Walker & del Moral, 2003), non-N-fixing plants responded positively, and produced more biomass in soils from N-fixing plants than in their own soil. This effect of soils from N-fixing plants on non-N-fixing plants in the young dune system was not only associated with the positive effect of abiotic soil conditioning (e.g., greater [NH_4^+]), but also with the positive effect of soil biota. This latter result does not support our first hypothesis that abiotic PSF is predominant in young soils. Conversely, N-fixing plants from the young dune system generally showed neutral PSF, which was different from that of co-occurring non-N-fixing plants, thus

supporting our second hypothesis that PSF depends on the type of plant functional group. Our third hypothesis was not fully supported by the absence of significant negative (biotic) PSF of similar magnitude in the old, severely P-impoverished soils. However, the shift in the direction of PSF experienced by non-N-fixing plants, from negative in young soils to neutral in the old soils, suggests that shifts in PSF depend on environmental context in terms of soil nutrient availability (Kardol et al., 2013; Lekberg et al., 2018; Manning et al., 2008). Collectively, our findings illustrate the importance of soil nutrient availability and the type of plant N-acquisition strategy in influencing the origin, strength and direction of PSF during long-term ecosystem development.

4.1 | Nitrogen-fixing plants facilitate non-nitrogen-fixing plants via biotic and abiotic plant-soil feedback

Soils from N-fixing plants had positive effects on the performance of co-occurring non-N-fixing plants via both biotic and abiotic PSF on the youngest chronosequence stage in which soil N availability strongly limits plant productivity (Hayes et al., 2014; Laliberté et al., 2012). The facilitative effects of N-fixing plants on non-N-fixing plants during early primary succession have been widely observed, and is generally attributed to their capacity to acquire atmospheric N through symbiotic N fixation and increasing soil N availability (Bellingham et al., 2001; Walker & del Moral, 2003). Indeed, we found greater [NH_4^+] in soils conditioned by N-fixing plants than by co-occurring non-N-fixing plants from the young dune system. However, we found that soil biota was an additional important factor, because biotic soil conditioning by N-fixing plants in the young dune system also had a positive effect on co-occurring non-N-fixing plants. The positive biotic effect experienced by non-N-fixing plants in soils from N-fixing plants might be explained by an accumulation of beneficial mycorrhizal fungi (Albornoz et al., 2016; Azcón-Aguilar & Barea, 1997; Smith & Read, 2008), plant growth-promoting rhizobacteria (Berendsen, Pieterse, & Bakker, 2012; García-Fraile et al., 2012; Lugtenberg & Kamilova, 2009; Yong, Letham, Wong, & Farquhar, 2014), or decomposers (e.g., de Deyn & van der Putten, 2005; Knops, Bradley, & Wedin, 2002). These microorganisms could promote plant growth, acquisition of limiting nutrients (e.g., iron, zinc, N; Laliberté et al., 2012), or pathogen defence in

young soils. Therefore, we showed that a combination of biotic and abiotic factors was involved in generating the positive effects of N-fixing plants on non-N-fixing plants in young soils, which illustrates the importance of simultaneously considering both abiotic and biotic PSF (Bardgett & Wardle, 2010; Bezemer et al., 2006; van der Putten et al., 2013).

4.2 | Differences in plant-soil feedback between plant nitrogen-acquisition strategies

Our second hypothesis that the direction of PSF depends on plant functional group was supported by the difference in the neutral PSF measured in N-fixing plants from the young, N-poor dune system versus the negative PSF in co-occurring non-N-fixing plants. The absence of abiotic PSF among common N-fixing species from the young dune system in soils from co-occurring heterospecific N-fixing plants suggests that abiotic soil conditioning by these N-fixing species is similar. In addition, despite the generally greater soil [NH_4^+] in young soils conditioned by N-fixing plants than in soils from co-occurring non-N-fixing plants, N-fixing plants from the young dune system showed no abiotic PSF when grown in sterilised soils from co-occurring non-N-fixing plant species. The absence of the predicted positive abiotic PSF of N-fixing plants from the young dune system in soils from co-occurring non-N-fixing plants might be explained by the tendency of N-fixing legumes to possess greater N demands relative to co-occurring non-N-fixing plants (McKey, 1994). As such, to detect a significantly greater investment in growth by N-fixing plants in young soils that will achieve the predicted positive PSF, a substantially greater amount of plant-available soil [N] than that measured in young soils beneath N-fixing plants may be required. Further, the absence of biotic PSF experienced by N-fixing plant species in young soils from heterospecific N-fixing plants is in line with previous studies (e.g., Birnbaum, Bissett, Thrall, & Leishman, 2014, 2016; Birnbaum & Leishman, 2013). Together, our results showing differences in biotic and abiotic PSF responses between N-fixing plants and co-occurring non-N-fixing plants from the young dune system demonstrate the dependency of PSF on the type of plant functional group or trait (Baxendale et al., 2014; Cortois, Schröder-Georgi, Weigelt, van der Putten, & de Deyn, 2016; Teste et al., 2017).

420 **4.3 | Plant-soil feedback in old, severely phosphorus-impoverished soils**

421 The shift in the direction of PSF experienced by non-N-fixing plants from negative to neutral with
422 increased soil age partly supports our third hypothesis that PSF depends on soil nutrient availability.
423 The absence of feedback in old soils conditioned by N-fixing plants might be linked to increased P
424 constraints on symbiotic N fixation (Hartwig, 1998; Raven, 2012) and legume productivity (Laliberté
425 et al., 2012) in these weathered, severely P-impoverished soils. The similar soil N inputs from the
426 leaf and root litter of N-fixing and non-N-fixing plants in the old soils (e.g., due to limited N fixation
427 by N-fixing plants) might reduce the ability of N-fixing plants to influence the performance of co-
428 occurring non-N-fixing plants via abiotic soil conditioning. In turn, similar soil N inputs by N-fixing and
429 non-fixing plants in the old soils might also favour soil microbial communities that exert similar
430 effects on the studied N-fixing and non-N-fixing species. Thus, the shift in the direction of feedback
431 experienced by non-N-fixing plants between young and old soils shows that PSF might depend on
432 the environmental context in terms of soil nutrient availability.

433 We did not find support for the hypothesised convergent shifts in PSF towards negative
434 biotic, pathogen-mediated PSF of similar magnitude in the old, severely P-impoverished dune
435 system. The absence of negative biotic PSF in non-N-fixing plants from the old, severely P-
436 impoverished dune system might be due to the strong impact of soil biota on plant growth in these
437 soils (Figures S1, S2 and S3). For example, the negative effect of soil biota on the growth of two (out
438 of three) non-N-fixing species examined in the old dune system (Figure S1) might be explained by
439 many plant species in these P-impoverished soils having root traits that enhance P acquisition (e.g.,
440 high specific root length, high root hair density) which trade off against pathogen defence (Laliberté
441 et al., 2015; Lambers et al., 2018; Newsham et al., 1995). This might increase plant susceptibility to a
442 broad range of non-host-specific soil-borne pathogens which could outweigh the potential negative
443 effects of host-specific pathogens.

444 On the other hand, the absence of negative biotic PSF in N-fixing plant species in old,
445 severely P-impoverished soils might be related to their similar positive responses to soil biota (Figure

S1). These legume species likely possess the ability to form effective symbiotic relationships with a range of N-fixing rhizobia that are ubiquitous in unsterilised soils (Birnbaum, Bissett, Teste, & Laliberté, 2018; Birnbaum et al., 2016; Checcucci, DiCenzo, Bazzicalupo, & Mengoni, 2017). Thus, symbiotic associations between N-fixing rhizobia and legumes might compensate for the potential negative effects of soil-borne pathogens if they enable greater investment of N in plant growth or pathogen defence (Menge & Chazdon, 2016; Menge, Levin, & Hedin, 2008; Vitousek & Field, 1999).

Understanding the full implications of our results, with regard to the shifts in PSF along the chronosequence and plant species coexistence mechanisms on species-rich, severely P-impoverished older soils, would warrant further research for three reasons. First, only common species from two plant functional groups (N-fixing and non-N-fixing) were examined in the present study, which would only have accounted for a subset of the range of PSF present among the diverse spectrum of plant functional groups in species-rich plant communities on these old, severely P-impoverished soils (e.g., Lambers et al., 2014; Zemunik et al., 2015). In particular, plant functional groups associated with key belowground nutrient-acquisition traits of AM, ECM, and non-mycorrhizal cluster roots require further attention (Lekberg et al., 2018; Revillini et al., 2016; Teste et al., 2017; Zemunik et al., 2015). Second, the underlying mechanisms of how various groups of soil microorganisms (e.g., N-fixing rhizobia, plant growth-promoting rhizobacteria, and soil-borne pathogens) influence PSF in this study remain something of a 'black box'. Third, our chronosequence approach does not allow strong inference of the causal mechanisms between the observed shifts in PSF and soil N and P availability (Walker et al., 2010). This is because differences in other abiotic and/or biotic factors along the chronosequence not considered in our study might also influence PSF. However, this chronosequence and its soils have been extensively studied (e.g., Abrahão, Ryan, Laliberté, Oliveira, & Lambers, 2018; Albornoz et al., 2016; Hayes et al., 2014; Laliberté et al., 2012, 2014; Teste, Veneklaas, Dixon, & Lambers, 2014; Turner & Laliberté, 2015; Zemunik et al., 2015, 2016), enabling us to adopt a sampling design that maximises variation in soil age (i.e. soil chemistry; in particular, soil fertility, N and P availability) while minimising variation in other factors (e.g.,

climate, topography). Therefore, to further advance our understanding of PSF during long-term ecosystem development, future studies should focus on PSF experiments involving soil nutrient manipulation, or test PSF responses along other long-term soil chronosequences. Further, future studies should also examine a greater number of plant functional groups in terms of nutrient-acquisition strategies and the soil microorganisms that influence PSF.

5 | CONCLUSIONS

Our results show that the shifts in the direction of abiotic and biotic PSF are context-dependent with respect to the type of plant N-acquisition strategy and soil nutrient availability. Our results also highlight the importance of considering both longer-term abiotic and shorter-term biotic PSF. In particular, the negative PSF experienced by non-N-fixing plants in the young, N-poor dune system was not only attributable to positive effects of abiotic soil conditioning by N-fixing plants, but also involved biotic soil conditioning. Overall, our study highlights the importance of considering environmental context and changes in both abiotic and biotic soil properties mediated by plants when studying PSF, which advances our mechanistic understanding of PSF in long-term ecological processes (Kardol et al., 2013; Lekberg et al., 2018; Smith-Ramesh & Reynolds, 2017; van der Putten et al., 2013). It also allows us to better appreciate how shifts in soil nutrient availability under future global change scenarios (e.g., increasing N deposition; Vitousek et al., 2013) may alter the direction of PSF and the consequences of this for the functioning of terrestrial ecosystems.

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501 G.K.P., P.K., D.A.W. and E.L. designed research; G.K.P., B.L.T. and E.L. performed research; G.K.P. and
502 E.L. analysed data; G.K.P., H.L., P.K., B.L.T., D.A.W. and E.L. wrote the paper. All authors contributed
503 critically to the drafts and gave final approval for publication.

504 **DATA ACCESSIBILITY**

505 Data available from the Dryad Digital Repository https://dx.doi.org/10.5061/dryad.4tc6292 (Png et
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SUPPORTING INFORMATION

Additional supporting information can be found in the online version of this article:

TABLE S1 Chemical properties of soil (0 - 20 cm depth) conditioned by common nitrogen (N)-fixing and non-N-fixing plants from two dune systems of contrasting soil ages along the Jurien Bay dune chronosequence

TABLE S2 Provenances of seeds and softwood cuttings of plants used in this study

TABLE S3 Results of three-factor linear mixed-effects model fitted with and without intercept terms to test for the effects among the factors of nitrogen (N)-fixing plant functional group type, plant functional group soil origin, soil age and their interactions on overall plant-soil feedback effects

TABLE S4 Results of three-factor linear mixed-effects model fitted with and without intercept terms to test for the effects among the factors of nitrogen (N)-fixing plant functional group type, plant functional group soil origin, soil age and their interactions on biotic plant-soil feedback effects

TABLE S5 Results of three-factor linear mixed-effects model fitted with and without intercept terms to test for the effects among the factors of nitrogen (N)-fixing plant functional group type, plant functional group soil origin, soil age and their interactions on abiotic plant-soil feedback effects

TABLE S6 Results of linear mixed-effects models of individual nitrogen (N)-fixing and non-N-fixing plant species from the young and old dune systems to test for differences between the general effects of biotic and abiotic soil treatments on plant growth

TABLE S7 Results of linear mixed-effects models of individual nitrogen (N)-fixing and non-N-fixing plant species from the young dune system to test for the effects of soil treatment, species soil origin and their interactions on plant growth

TABLE S8 Results of linear mixed-effects models of individual nitrogen (N)-fixing and non-N-fixing plant species from the old dune system to test for the effects of soil treatment, species soil origin and their interactions on plant growth

FIGURE S1 General effect of the soil inoculation treatment on the growth of individual nitrogen (N)-fixing and co-occurring non-N-fixing plant species compared with that in gamma-sterilised soils (abiotic soil treatment) from the young and old dune systems.

FIGURE S2 Total growth of individual nitrogen (N)-fixing and non-N-fixing plants grown in overall (field-untreated), biotic (inoculated) and abiotic (gamma-sterilised) soils conditioned by co-occurring N-fixing or non-N-fixing plants from the young dune system.

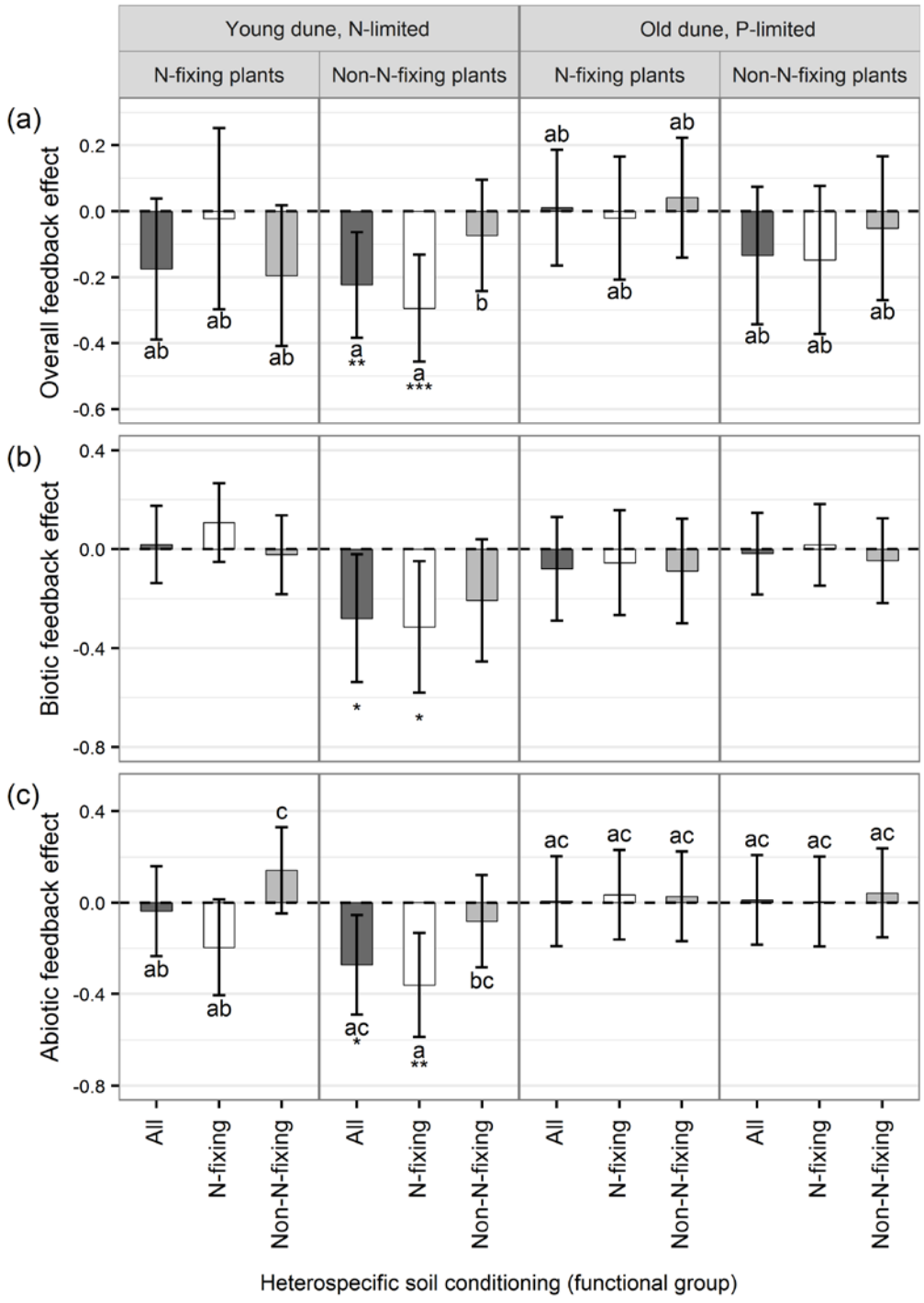
FIGURE S3 Total growth of individual nitrogen (N)-fixing and non-N-fixing plants grown in overall (field-untreated), biotic (inoculated) and abiotic (gamma-sterilised) soils conditioned by co-occurring N-fixing or non-N-fixing plants from the old dune system.

TABLE

TABLE 1 Common nitrogen (N)-fixing and non-N-fixing tree or shrub species selected from two dune systems of contrasting soil ages along the >2 million-year Jurien Bay dune chronosequence based on survey data of Zemunik et al. (2015, 2016). Likely soil nutrients limiting plant productivity are based on Laliberté et al. (2012). Nutrient-acquisition strategies of non-N-fixing species and, if applicable, degree of mycorrhizal root colonisation are based on Zemunik et al. (2015). AM, arbuscular mycorrhizal; ECM, ectomycorrhizal; NM, non-mycorrhizal

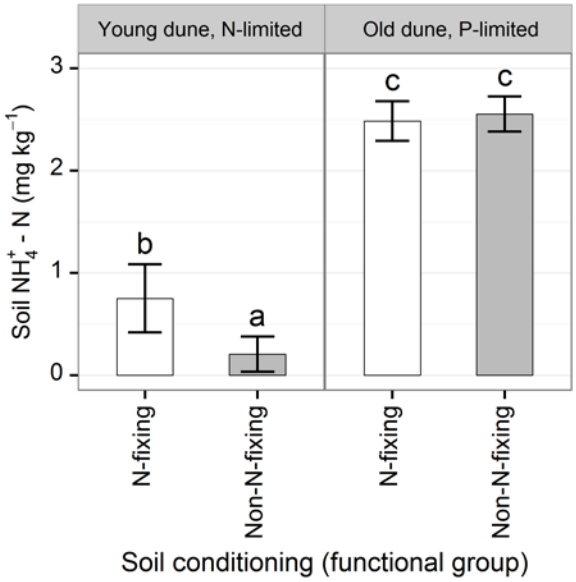
Dune system (soil age)	Likely soil nutrient limiting plant productivity	Selected tree/shrub species		
		N-fixing species	Non-N-fixing species	Nutrient-acquisition strategy of non-N-fixing species
Quindalup Young (Holocene, <0.1 ka)	Nitrogen	*Acacia rostellifera*	*Olearia axillaris*	AM (moderate)
		Acacia cyclops	*Scaevola crassifolia*	AM (low-moderate); ECM (moderate)
		Acacia truncata	*Spyridium globulosum*	AM (moderate); ECM (moderate)
Spearwood West (Middle Pleistocene, >120 ka)	Phosphorus	*Acacia rostellifera*	*Banksia leptophylla*	NM (cluster roots)
		Acacia spathulifolia	*Calothamnus quadrifidus*	ECM (moderate)
		Gompholobium tomentosum	*Melaleuca systena*	AM (low); ECM (low)

764 **FIGURES**



765

766 **FIGURE 1** Plant-soil feedback of nitrogen (N)-fixing and non-N-fixing plant functional groups grown in (a)
767 overall (field-untreated), (b) biotic (inoculated), and (c) abiotic (gamma-sterilised) soils conditioned by co-
768 occurring heterospecific plants in general (all heterospecific species), N-fixing and non-N-fixing plants from two
769 dune systems of contrasting soil ages along the Jurien Bay dune chronosequence (Table 1). Each type of
770 feedback was calculated as $\log_{10}$(biomass of a plant species grown in conspecific conditioned soil/average
771 biomass of plant species grown in soils conditioned by all or N-fixing or non-N-fixing heterospecific species).
772 Bars are means and error bars represent 95% confidence intervals from a linear mixed-effects model for each
773 panel (Tables S3, S4 and S5). *, **, *** indicate significant feedback at $P \leq 0.05$, 0.01, or 0.001 (Tables S3, S4
774 and S5). Different letters among soil conditioning treatments indicate significant differences among means
775 within each panel (*post hoc* Tukey tests, $P \leq 0.05$); letters are not included when means did not differ.



776

777 **FIGURE 2** Ammonium (NH_4^+) content of soils collected to 20 cm depth beneath three nitrogen (N)-fixing and
778 three co-occurring non-N-fixing plant species from each of two dune systems of contrasting soil ages along the
779 Jurien Bay dune chronosequence (Table 1). Bars are means and error bars represent 95% confidence intervals
780 from a linear mixed-effects model (Table S1). Different letters among soil conditioning treatments indicate
781 significant differences among means (*post hoc* Tukey test, $P \leq 0.05$).