

What drives retrogressive succession? Plant strategies to tolerate infertile and poorly drained soils

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Summary

1. During retrogressive succession, vegetation shifts from taller forests with higher species richness to shorter woody communities of lower diversity. Studies of soil chronosequences have emphasized the role of phosphorus (P) depletion in driving these changes in community structure and composition, but neglected the possible role of poor drainage which is often associated with the oldest sites.

2. We used a fully factorial pot experiment to investigate the effects of soil water conditions and P availability on seedling relative growth rate and survival of 11 woody species associated with a 290 000-year chronosequence in southern New Zealand. Species were chosen to represent three stages of the chronosequence: young P-rich soils; intermediate-age P-depleted soils; and old P-depleted, waterlogged soils. Plants were grown in soils taken from youngest and oldest sites of the chronosequence; half the pots were freely drained whilst the other half were waterlogged.

3. Species associated with intermediate-age soils were typical ‘stress-tolerators’; those of the youngest and oldest sites were faster growing and more responsive to nutrient availability. Only the latter showed tolerance of waterlogging. Specific root length and adventitious-root production were important determinants of species’ responses to soil water conditions and nutrient supply.

4. Our study highlights that soil waterlogging and P depletion interact to influence relative growth rate and survival; P depletion is not the sole driver of ecosystem changes in the retrogressive stages of the soil chronosequence. We also show that some species associated with our retrogression did not necessarily conform to conventional views on ‘stress-tolerance’, but were well adapted to poor drainage.

Key-words: adventitious roots, gymnosperm, New Zealand, root:shoot ratio, specific root length, stress-tolerator, temperate rain forest, Waitutu chronosequence

Introduction

The Walker & Syers (1976) model of soil phosphorus (P) transformations through time has become the paradigm of terrestrial biogeochemistry and a central concept in ecological succession theory (Wardle *et al.* 2008). Walker & Syers (1976) proposed that P is most available to plants and microbes in early stages of primary succession, when P contained in minerals such as calcium apatite dissolves to produce inorganic phosphates available for plant uptake. During the process of pedogenesis much of the P becomes inaccessible to organisms, because it is occluded in allophane and hydrous oxides of iron and aluminium, or incor-

porated into recalcitrant organic matter (Crews *et al.* 1995). These changes have been associated with reductions in P recycling from litter decomposition [as microbial communities are hampered by P limitation (Cleveland, Townsend & Schmidt 2002)] and with P limitation of plant growth (Vitousek & Farrington 1997). The model of Walker & Syers has been supported by many papers focusing on ecosystem succession (Chapin *et al.* 1994; Crews *et al.* 1995; Herbert & Fownes 1995; Vitousek 2004; Parfitt *et al.* 2005).

Tree growth and forest biomass are often observed to decline in the oldest sites of soil chronosequences, and P limitation is usually given as the explanation (Wardle, Walker & Bardgett 2004). However, some of the study sites selected for these ‘retrogressive successions’ are also poorly drained, because impermeable iron pans form under high-rainfall conditions, impeding soil drainage and initiating paludification (Kitayama *et al.* 1997). For example, of the six sites chosen

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by Wardle, Walker & Bardgett (2004) for a global comparison of chronosequences, the oldest sites on the Waitutu, Franz Josef and Glacier Bay chronosequences were oligotrophic bogs [see Norton (1989), Chapin *et al.* (1994) and Mark *et al.* (1988) for descriptions of these sites]. Therefore it is plausible that poor drainage contributes to retrogressive succession, given that soil waterlogging is also associated with canopy height reductions and increased occurrence of shrubs (Kitayama *et al.* 1997; Crawford, Jeffree & Rees 2003; Coomes *et al.* 2005). However, the relative contributions that P depletion and soil waterlogging make to the survival and growth of species associated with old soils remains unresolved.

The commonly held view is that species associated with nutrient poor soils are inherently slow growing 'stress-tolerators' (*sensu* Grime 1977), characterized by low phenotypic plasticity and long-lived organs that maximize acquisition and conservation of nutrients (Grime 1979; Chapin 1980) at a cost to growth (Aerts & Van der Peijl 1993). The paradigm is that such 'stress-tolerators' are relatively unresponsive to greater nutrient supply and are outcompeted on nutrient-rich sites (Chapin 1980). However, adaptations to efficient nutrient acquisition may be different to those required for survival in strongly oxygen-limited soils. Plants associated with nutrient-poor environments often have high root:shoot ratios, associated with greater carbon investments to soil exploration, whereas waterlogging tends to favour low root:shoot ratios primarily because root respiration rates are limited by oxygen supply (Voeseinek, Blom & Pouwels 1989; Rubio & Lavado 1999). Secondly, there is some evidence that species from nutrient-poor sites have low specific root length (SRL, amount of root length per unit root mass) (Fitter 1991) associated with thicker roots with low respiration rates and long lifespans (Reich *et al.* 1998; Comas & Eissenstat 2004), whereas waterlogging favours highly porous root systems (Armstrong *et al.* 1991) with low tissue density and therefore high SRL, as SRL is affected by root diameter and tissue density (Loreti & Oesterheld 1996).

In this experiment, we evaluated the responses of 11 species from three stages of the Waitutu Chronosequence to soil nutrient limitation and waterlogging to look at the relative importance of these two stresses on their growth and survival. We asked the following questions: (i) is waterlogging more detrimental to the growth and survival of plants in nutrient-poor soils than in nutrient-rich soils? (ii) are the effects of waterlogging and nutrient-limitation on the species' growth and survival correlated with changes in root:shoot ratio and SRL? and (iii) will species associated with intermediate and old soils on the Waitutu chronosequence show the characteristics of classic stress tolerators?

Materials and methods

STUDY SPECIES AND STUDY AREA

All soils and seedlings used in the shade-house experiment were collected at the Waitutu Chronosequence, in Waitutu Ecological

Region, Fiordland National Park, on the south-west coast of the South Island, New Zealand (46.4°S, 167.2°E). The chronosequence consists of a series of marine terraces intersected by watercourses flanked with alluvial deposits (Mark *et al.* 1988). Alluvial terraces were formed within the last 24 000 years (24 kyr), and some are periodically replenished with alluvium. Marine terraces were uplifted between 80 and 290 kyr BP. We chose three types of site to represent the chronological and nutrient gradient along the terrace sequence: 'young alluvial sites' on the alluvial terraces have soils relatively plentiful in total P ($87 \pm 7.9 \text{ g m}^{-2}$, taken from Coomes *et al.* 2005); 'intermediate-age' sites are on marine terraces uplifted 80–120 kyr BP with soils deficient in P ($11 \pm 0.36 \text{ g m}^{-2}$); and the 'oldest sites', uplifted 290 kyr BP, with soils also P-deficient ($8 \pm 0.5 \text{ g m}^{-2}$). These sites also represent a gradient in terms of soil drainage; measurements of the water table indicate that during 4 months of the year the water table is within 0–20 cm of the surface just 3% of the time on alluvial soils, 14% on intermediate-age sites, but persisted 44% of the time on oldest sites (Coomes *et al.* 2005). These oldest and most poorly drained sites are regarded as being in the 'retrogressive' phase of succession because the vegetation is shorter than that on younger sites (Wardle, Walker & Bardgett 2004).

Species chosen for the experiment included both broadleaved evergreen conifers and angiosperms, tall- as well as sub-canopy species (Table 1). They were selected on the basis of basal area estimates from permanently marked plots, i.e. those most common on the youngest soils, on intermediate-age soils and on the oldest soils (see Coomes *et al.* 2005). From the alluvial sites we chose the two dominant canopy species, *Nothofagus menziesii* and *Weinmannia racemosa* and four sub-canopy species. From intermediate-age sites we included the four dominant canopy trees: three species of conifer (Podocarpaceae) and *Metrosideros umbellata* (Myrtaceae). From the oldest sites we could only work with two of the four dominant species, because the other two spread vegetatively and very rarely produce seedlings. Henceforth, we refer to these groupings of species as 'habitat associations'.

SEEDLING AND SOIL COLLECTION

Seedlings 4–12 cm in height (depending on the species) were carefully excavated from forests within the Waitutu Chronosequence, and only those that were ground-rooted were chosen. Before transplanting the seedlings, all forest soil was removed from the roots by gentle washing to avoid root damage. We extracted soils from a P-rich alluvial site and from one of the P-depleted oldest terraces, taking a total of 740 kg of soil from within 20 cm of the surface of each site. Soils were transported to the Landcare Research nursery near Christchurch, New Zealand.

EXPERIMENTAL DESIGN

Seedlings were grown in one of the two soil types (P-rich and P-depleted), in combination with two states of soil water (waterlogged and non-waterlogged). Our factorial experiment used a randomized block design, with four treatments and 11 species, replicated in 13 blocks.

Seedlings were individually planted in 1.5 L pots (15 cm tall), half the pots being randomly assigned to P-rich soil and half to P-depleted soil. For each species, two plants growing in P-rich soil and two growing in P-depleted soil were randomly allocated to each of 13 large plastic tanks (5 m × 3 m), which acted as blocks. Tanks were arranged within a shade house made from green cloth (intercepting

Table 1. List of species used in the waterlogging experiment. Species' relative abundance along the Waitutu Chronosequence on young alluvial terrace (1), intermediate-age sites (2), and old marine terraces (3) is expressed as c: common, r: rare and a: absent (adapted from Coomes *et al.* 2005)

Species	Family	Group	Dominance along the sequence			Habitat association
			1	2	3	
<i>Coprosma foetidissima</i> †‡	Rubiaceae	Angiosperm	r	r	a	Youngest soil
<i>Dacrydium cupressinum</i>	Podocarpaceae	Gymnosperm	r	c	a	Intermediate soil
<i>Fuchsia excorticata</i> ‡	Onagraceae	Angiosperm	c	a	a	Youngest soil
<i>Griselinia littoralis</i>	Cornaceae	Angiosperm	c	r	a	Youngest soil
<i>Leptospermum scoparium</i> ‡	Myrtaceae	Angiosperm	a	a	c	Oldest soil
<i>Metrosideros umbellata</i>	Myrtaceae	Angiosperm	a	c	a	Intermediate soil
<i>Nothofagus menziesii</i>	Nothofagaceae	Angiosperm	c	c	a	Youngest soil
<i>Nothofagus solandri</i> *†	Nothofagaceae	Angiosperm	a	c	c	Oldest soil
<i>Podocarpus totara</i>	Podocarpaceae	Gymnosperm	r	c	a	Intermediate soil
<i>Prumnopitys ferruginea</i>	Podocarpaceae	Gymnosperm	r	c	a	Intermediate soil
<i>Pseudowintera colorata</i> ‡	Winteraceae	Angiosperm	c	r	a	Youngest soil
<i>Weinmannia racemosa</i>	Cunoniaceae	Angiosperm	c	c	a	Youngest soil

*var. cliffortioide.

‡Shrub or small tree.

†Common on alluvial and terrace sites, but recorded as rare because it attains low basal area and rarely reaches the diameter needed for recording in the inventory plots.

80% of incoming radiation, as determined by quantum sensors on a cloudy day) and left for 3 months to allow seedlings to recover from stresses of transportation and potting. During this time any seedlings that died were substituted with spares kept in the shade house.

In late March 2002, the four seedlings per species within each tank were allocated to four treatments: fertile soil + well drained (hereafter FD); infertile soil + well drained (ID); fertile soil + waterlogged (FW); and infertile soil + waterlogged (IW). Seedlings allocated to well drained treatments were raised off the tank floor by 12.5 cm, whereas those allocated to waterlogging treatments remained on the floor. The spacing of seedlings was sufficient to ensure that the raised seedlings did not shade their neighbours. Tanks were filled up to 12.3 cm with rainwater and this level was maintained with regular watering, at least twice a week. To prevent overflowing of the tanks a series of holes were drilled in their sides at 12.3 cm height, allowing excess water to drain away. Seedlings were re-randomized every 2 months within the water tanks to avoid the effects of any gradients of irradiance in the shade house, and remained in the shade house for 2 years. During this time, seedling survival was monitored every month. A seedling was considered dead if no green leaves were present.

We calculated the dry weight of 8–10 spare seedlings per species at the start of the watering treatments, and used these weights as initial values when calculating growth rates. At harvest, all seedlings were removed from the pots and washed until no soil was left on the roots. The presence of any adventitious roots (those that grew above the white mark left when we took initial measurements) was noted on harvested plants. Woody and non-woody roots were harvested together and a sample of non-woody roots was taken from five randomly selected seedlings (five per species per treatment; $n = 220$) for root length measurements; these were digitized at 600 dpi as a grey-scale image. Images were converted to black and white by choosing a threshold value equally placed between the mean brightness of the root and the background. Each root in the image was reduced to single-pixel thickness using Hilditch's algorithm (Hilditch 1969) and vectorized (Bell 1973) to produce a list of pixel locations along each

thinned root in the image (using Matlab version 6; The MathWorks Inc., Natick, USA). Root length (mm) was then calculated as the total perimeter of each root image divided by two, and SRL (g m^{-1}) as root length divided by dry weight (determined by oven-drying the samples for 3 days at 60 °C). Other seedling parts were also oven-dried and leaf, root, and stem dry weights recorded. The dry weights from root samples used for SRL calculation were included in the measurement of total root dry weight. Unfortunately, leaf area measurements taken at final harvest were not subsequently used, due to concerns over the reliability of the equipment used. Relationships between shoot, root, leaf, and plant weights were obtained by fitting standardized major axis lines to allometric relationships (i.e. \log_{10} – \log_{10} relationships). The advantage of this approach over traditional analyses of root:shoot ratio is that it takes into consideration any changes in allocation with plant size (Lensen, Menting & Van der Putten 2003). Differences in allometric relationships of plants growing in the four treatments were tested using likelihood ratio tests, on a species-by-species basis, using standardized major axis analyses (the *smatr* function in *R*; Warton & Weber 2002).

ANALYSES OF VARIANCE

Analyses of variance were used to test the effects of soil water conditions and soil fertility on relative growth rate and SRL. Relative growth rate in biomass (R_B) was calculated as $[(\log_e(\text{final biomass}) - \log_e(\text{initial biomass}))/2]$ (Cornelissen, Castro Diez & Hunt 1996) and used as the response variable. The explanatory variables included species identity, soil water conditions, soil fertility, the interaction between these, and a block term. We were interested in knowing whether differences among species were related to their habitat associations (e.g. whether species from intermediate-age soils were generally slow growing). To examine this point, we partitioned the variance associated with species into a habitat-association effect and residual effect, by replacing 'species' in the models with 'habitat association' and 'species within habitat' terms.

Results

SEEDLING RELATIVE GROWTH RATE

Relative growth rate (R_B) was greater in fertile than in infertile soils ($0.71 \pm 0.06 \text{ yr}^{-1}$ vs. $0.26 \pm 0.02 \text{ yr}^{-1}$) and was greater in well drained than in waterlogged soils ($0.66 \pm 0.05 \text{ yr}^{-1}$ vs. $0.31 \pm 0.03 \text{ yr}^{-1}$). Species varied a lot in R_B , and responded differently to the treatments (Fig. 1b,c). About 70% of variation among species was related to their habitat associations (species \times habitat association term contribute $SS = 23.5$ within the species term $SS = 32.8$; Table 2). In general, species from the intermediate-age sites were slower growing and less responsive to soil-nutrient supply than species from the youngest and oldest sites (Fig. 1b,c).

Some species associated with the young alluvial soils were intolerant of waterlogging, with the exception of *Coprosma* and *Pseudowintera* on fertile soils (Fig. 1c). Species associated with the intermediate-age sites were intolerant of waterlogging (Fig. 1c), while species associated with the oldest sites were tolerant of waterlogging, particularly on fertile soils (Fig. 1c). Species increased in biomass under soil waterlogging, particularly in fertile soils, which was reflected in the significant effect of the

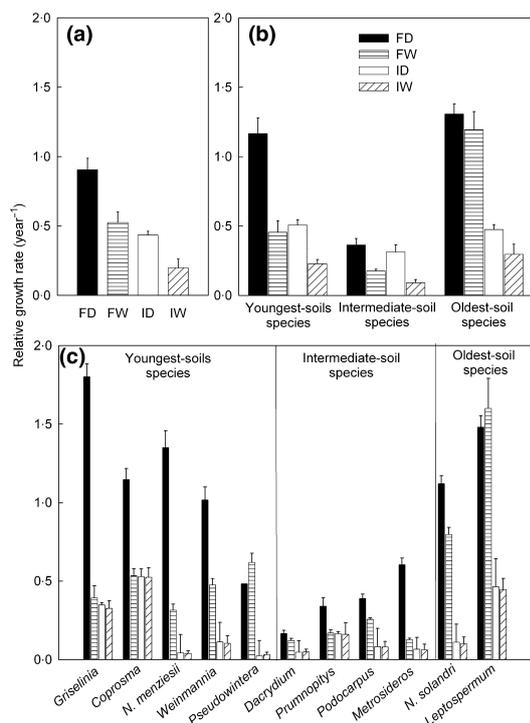


Fig. 1. Effects of soil water conditions (W = waterlogged, D = well drained) and nutrient availability (F = fertile soil, I = infertile soil) on mean relative growth rates in biomass (R_B). (a) All species pooled. (b) Species grouped according to habitat association: species associated with youngest alluvial soils (five species), intermediate-age soils (four species), and oldest soils (two species). (c) 11 individual species, ordered according to their natural distribution (species near the left are common to the youngest alluvial soils and towards the right to the oldest-soil marine terraces). Data shown are means ± 1 SE.

water \times species term. However, only 2% of the variance was related to species habitat association (Table 2), thus most of the variation can be attributed to species-specific responses. This is also reflected in the low percentage of variance (4%) within the water \times soil \times species term that was explained by species' habitat associations (Table 2). There was some indication of a trade-off between growth rate of species in FD soil and survival rates under waterlogged conditions. The trade-off was marginally significant in the case of FW soils ($\rho = 0.61$; $P = 0.04$) and not significant in the case of IW soils ($\rho = 0.59$; $P = 0.13$).

SPECIFIC ROOT LENGTH AND ROOT RESPONSES

Specific root length was greater for seedlings growing in fertile than in infertile soils ($43.5 \pm 2.87 \text{ m g}^{-1}$ vs. $32.9 \pm 2.17 \text{ m g}^{-1}$) and for seedlings growing in well drained than waterlogged soils ($43.3 \pm 2.64 \text{ m g}^{-1}$ vs. $33.0 \pm 2.45 \text{ m g}^{-1}$). The decrease in SRL was associated with waterlogging and infertile soils (Fig. 2a; Table 2). SRL varied greatly among species and treatments (Fig. 2b,c) and only 38% of variation among species was related to habitat associations, indicating that most of the variation occurs at the species level (Table 2). For species associated with the youngest sites SRL was highest in FD soils but reductions in SRL were not clearly associated with waterlogging as low fertility also reduced SRL (Fig. 2b). With species from intermediate-age soils variation in SRL cannot be clearly associated to a treatment, although there is some indication of reductions in SRL in waterlogged soils (Fig. 2b). A very different pattern is observed in the two species associated with the oldest sites, where SRL actually increased in waterlogged soils (Fig. 2b).

Across species, the effects of waterlogging on SRL seemed to vary with no general pattern emerging (Fig. 2c). Noteworthy is that within the water \times species term 47% of the variance was related to species habitat association (Table 2). However, within the water \times soil \times species term, only 7% of the variance was related to habitat association, which indicates that most of the variation is due to species idiosyncratic responses to soil waterlogging and nutrient availability (Table 2). Finally, we found a positive linear relationship between R_B and SRL for those seedlings growing in FD soils (Fig. 3), and a marginally significant relationship for seedlings on FW soils ($P = 0.05$) (Fig. 3).

Another measure of a species' potential to respond to waterlogging was the ability to produce adventitious roots. These roots were only observed on waterlogged seedlings, and on only six of the 11 species (Table 3). Mean numbers of adventitious roots were similar on fertile and infertile soils (IW = 8.0 ± 0.79 vs. FW = 7.7 ± 0.73 ; $t = 0.78$; d.f. = 80; $P = 0.21$), thus waterlogging and not nutrient availability promotes their presence. Cross-species correlation analysis showed a significant positive relationship between survival and proportion of seedlings with adventitious roots in waterlogged soils (Pearson's correlation = 0.78; $P < 0.01$).

Table 2. Analyses of variance of (a) relative growth rate (R_B) and (b) specific root length (SRL) of seedlings in a fully factorial pot experiment in which 11 species were grown in fertile and infertile soil ('soil' effect), in well drained and poorly drained pots ('water' effect). Species were grouped into three habitat associations, reflecting the type of forest in which they were most commonly found. The models explained 96% and 94% of the variation in growth and specific root length respectively

	Biomass growth			Specific root length		
	d.f.	SS	F	d.f.	SS	F
Soil	1	20.4	2106***	1	0.3	20***
Water	1	11.41	1173***	1	1.0	78***
Soil × water	1	0.5	56***	1	1.5	109***
Species	10	32.8	338***	10	15.0	114***
(Habitat association)	2	23.5	1209***	2	5.8	217***
Soil × Species	10	12.1	125***	10	1.8	13**
(Soil × Habitat association)	2	10.0	518***	2	0.2	9**
Water × Species	10	10.1	104***	10	3.5	26***
(Water × Habitat association)	2	2.9	153***	2	1.8	64***
Water × Soil × Species	10	6.3	65***	10	3.0	22***
(Water × Soil × Habitat assoc.)	2	1.7	84***	2	0.2	6***
Block effect	12	0.6		12	0.7	
Residual	416	4.1		163	2.2	

d.f., degrees of freedom; SS, Sum of Squares.

* $P < 0.01$; ** $P < 0.001$; *** $P < 0.0001$.

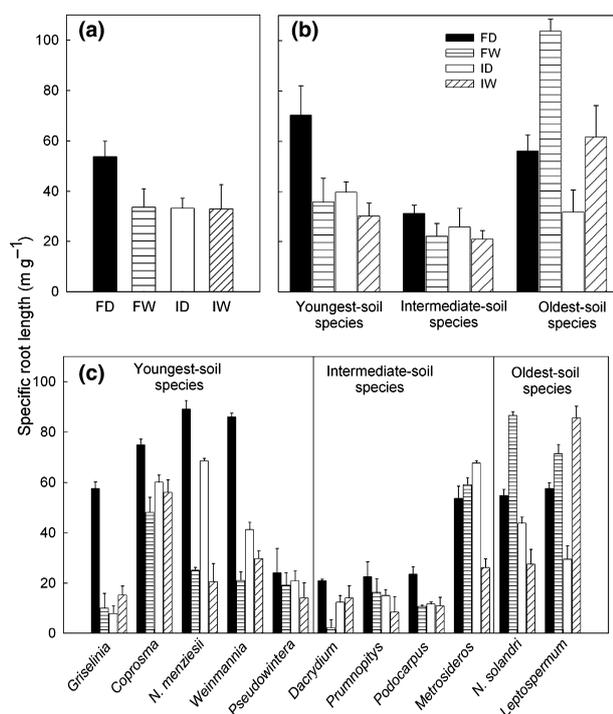


Fig. 2. Effects of soil water conditions (W = waterlogged, D = well drained) and nutrient availability (F = fertile soil, I = infertile soil) on specific root length (SRL). (a) All species pooled. (b) Species grouped according to habitat association: species associated with youngest alluvial soils (five species), intermediate-age soils (four species), and oldest soils (two species). (c) 11 individual species, ordered according to their natural distribution (species near the left are common to the youngest alluvial soils and towards the right to the oldest-soil marine terraces). Data shown are means \pm 1 SE.

BIOMASS ALLOMETRY

Allometric relationships of root:shoot ratio varied in intercept ($L = 36.1$, $P < 0.0001$) but not in slope ($L = 2.5$,

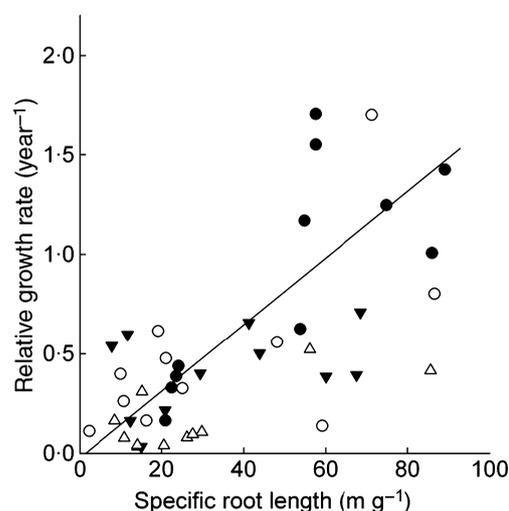


Fig. 3. Relationship between mean relative growth rate in biomass (R_B) and specific root length of 11 species grown in the following treatments: (●) fertile well drained, (○) fertile waterlogged, (▲) infertile well-drained, and (△) infertile waterlogged. The line represents significant least square regression for fertile well drained soils; ($R = 0.69$; $F_{1,9} = 20.6$; $P < 0.001$) and a marginally significant for fertile waterlogged soils ($R = 0.37$; $F_{1,9} = 4.71$; $P = 0.05$) (other correlations are not significant).

$P = 0.46$) among the four treatments when data were pooled across species. The common slope estimate was indistinguishable from a value of 1 (0.95, 95% CI = 0.90–1.005) indicating that root:shoot ratios were invariant with respect to seedling size. Root : shoot ratios estimated by back-transforming intercept estimates (i.e. $10^{\text{intercept}}$) decreased among the four treatments as follows: ID > IW > FW > FD (0.52, 95% CI = 0.48–0.56, 0.46, 95% CI = 0.41–0.51, 0.43, 95% CI = 0.38–0.48 and 0.31, 95% CI = 0.27–0.33 respectively). Analyses on a species-by-species basis yield similar trends. Species

Table 3. Seedling survival (number surviving out of 13) and presence of adventitious roots for combinations of fertile (F) and infertile (I), waterlogged (W) and well drained (D) soils. Species are ordered according to their natural distribution on a soil chronosequence

Species	Number of surviving seedlings					χ^2 (d.f. = 3)	Number of plants with adventitious roots				
	FD	FW	ID	IW	FD		FW	ID	IW	χ^2 (d.f. = 3)	
Associated with the youngest soil											
<i>G. littoralis</i>	13	13	13	13	1.92 NS	0	12	0	8	33.64***	
<i>P. colorata</i>	12	8	10	8	2.92 NS	0	5	0	0	10.51*	
<i>N. menziesii</i>	12	8	12	9	2.27 NS	0	0	0	0	0.85 NS	
<i>W. racemosa</i>	13	8	13	10	2.53 NS	0	5	0	3	26.88***	
<i>C. foetidissima</i>	13	7	12	11	2.76 NS	0	6	0	11	24.14***	
Associated with the intermediate-age soil											
<i>D. cupressinum</i>	13	7	12	8	3.63 NS	0	0	0	0	2.00 NS	
<i>P. ferruginea</i>	11	8	10	9	1.88 NS	0	0	0	0	4.21 NS	
<i>P. totara</i>	12	9	12	8	2.61 NS	0	0	0	0	5.34 NS	
<i>M. umbellata</i>	13	6	12	10	4.92 NS	0	2	0	4	14.50*	
Associated with the oldest soil											
<i>N. solandri</i> †	12	10	13	10	2.27 NS	0	0	0	0	5.14 NS	
<i>L. scoparium</i>	13	13	11	13	1.25 NS	0	13	0	12	46.30***	

NS, not significant.

* $P < 0.01$; ** $P < 0.001$; *** $P < 0.0001$.

†var. cliffortioides.

had higher root:shoot ratios when grown in infertile soil than in fertile soil, but only when well drained (Fig. 4a vs. b). Species had higher root:shoot ratios when waterlogged than when well drained, but only when grown in fertile soil (Fig. 4c vs. d). Species with high root:shoot ratios had low growth rate (R_B) under 'optimal' conditions (i.e. FD treatment; Pearson's correlation = -0.68 ; $t = 2.80$; d.f. = 9; $P = 0.02$), whereas species allocating more mass to roots had greater R_B under infertile-well drained soils (i.e. ID treatment; Pearson's correlation = 0.65 , $t = 2.56$, d.f. = 9, $P = 0.03$) and no relationship was found for the other treatments.

Allometric relationships of root versus leaf mass varied among treatments in slope ($L = 51.19$, $P < 0.0001$) and intercept ($L = 96.27$, $P < 0.0001$), so comparisons were made by predicting the root mass of seedlings with leaf biomass of 0.1 g for each treatment. We found that seedlings growing in the IW soil had the highest biomass of roots in relation to leaf mass (0.101, 95% CI = 0.114–0.095) followed by seedlings in FW soil (0.067, 95% CI = 0.085–0.049) and FD soil (0.051, 95% CI = 0.072–0.030), whilst seedlings in ID soil had the lowest ratio (0.016, 95% CI = 0.023–0.010) (Fig. 4b). Species varied in root:leaf ratio when grown in FD soil ($L = 24.41$, $P < 0.01$) and FW soil ($L = 31.93$, $P < 0.001$), but not when grown in ID soil ($L = 17.14$, $P = 0.07$) or IW soil ($L = 16.38$, $P = 0.08$).

SEEDLING SURVIVAL

Almost all seedlings grown in well drained soil survived until the end of the experiment (cross-species mean, $93.5 \pm 6.5\%$), and no differences were observed between FD and ID soils ($\chi^2 = 0.6$; d.f. = 1; $P = 0.45$). Seedling survival was adversely affected by waterlogging (GW $\chi^2 = 55.2$; d.f. = 1; $P < 0.0001$) and in contrast to our hypothesis, survival of waterlogged seedlings was lower on

fertile soils than on infertile soils (GW $\chi^2 = 68$; d.f. = 3; $P < 0.0001$) (Table 3).

Discussion

INTERACTIONS BETWEEN WATERLOGGING AND NUTRIENT SUPPLY ON SEEDLING RELATIVE GROWTH RATE AND SURVIVAL

Waterlogging inhibits below-ground respiration and thereby reduces growth and survival of plants that lack mechanisms for supplying oxygen to roots (Drew, He & Morgan 2000). Mechanisms for root oxygenation include: the formation of adventitious roots (Jackson & Drew 1984), which we observed in six out of the 11 species; the reduction of root:shoot ratio (Voesenek, Blom & Pouwels 1989; Rubio *et al.* 1997), which was observed in only three of the 11 species; and root aerenchyma, which was not examined in our study but has been reported in *Leptospermum* (Cook, Mark & Shore 1980). We hypothesized that waterlogging would be particularly detrimental to seedlings growing in nutrient-poor soils, because tolerance of anoxia requires different morphological and/or physiological adaptations to tolerance of nutrient deficiency. The growth responses of seedlings generally support this hypothesis: we found that the combination of waterlogging and low-nutrient soils had the greatest impact on plant growth for nine of the 11 species examined, regardless of habitat association on the chronosequence (Fig. 1c). However, the negative effects on plant growth of waterlogging and nutrient shortage did not translate into increased mortality over the course of the study; seedling mortality was higher on fertile than infertile waterlogged soils (55% vs. 45%) (Table 3). Perhaps plants growing on the fertile soils produced many fine roots prior to the imposition of waterlogging, an efficient strategy for acquiring nutrients in fertile soils

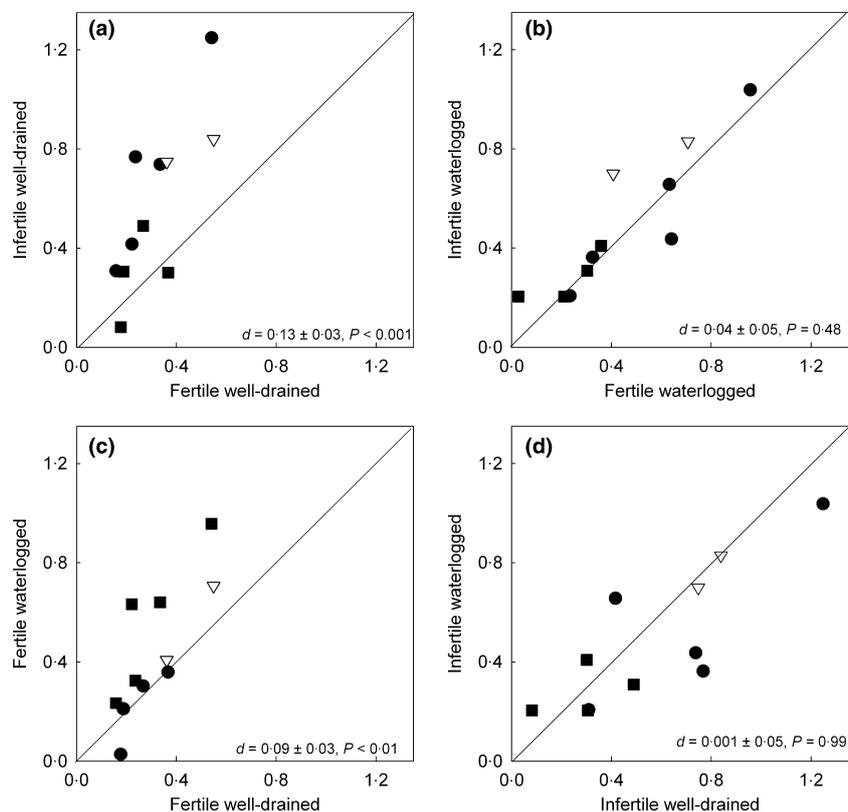


Fig. 4. Comparison of root:shoot ratios (back transformed intercepts of allometric relationships) of 11 species grown in: (a) nutrient-rich versus nutrient-poor well drained soils; (b) nutrient-rich versus nutrient-poor waterlogged soils; (c) waterlogged versus well-drained fertile soils; and (d) waterlogged versus well drained infertile soils. Tests of whether root:shoot ratio shifts were statistically significant are based on calculating the mean orthogonal distance of the points from the 1:1 line ($d \pm 1$ SEM) and using t -tests (see numbers in each panel). Symbols represent the habitat association of the species: (●) young alluvial soils, (■) intermediate-age soils and (▽) oldest soils.

(Fitter 1991), and these fine roots become an impediment once the soils turned anoxic.

CHANGES TO ROOT ALLOCATION AND SRL ASSOCIATED WITH NUTRIENT-SHORTAGE AND WATERLOGGING

We hypothesized that the effects of waterlogging and nutrient-limitation on the species' relative growth rate and survival will be correlated with changes in root:shoot ratio and SRL. Consistent with many previous studies, we found that in well drained soil most species responded to low nutrient availability by allocating more resources to root growth (Reich *et al.* 1998). The interactive effect of waterlogging and nutrient limitation produced a less clear picture. Under conditions of waterlogging, root:shoot ratio increased for the five species from the youngest sites when grown in fertile soil (Fig. 4c), but decreased in infertile soil (Fig. 4d). Similarly, slope comparisons of root:leaf revealed that species tended to reduce leaf and maintain high root biomass in ID soils, but reduce both leaf and root biomass in IW soils (Fig. S1). Previous studies on the impacts of waterlogging on root allocation report increases (Lenssen, Menting & Van der Putten 2003), decreases

(Rubio, Casasola & Lavado 1995; Rubio *et al.* 1997) and no impacts (Rubio & Lavado 1999), so no consistent pattern emerges.

Decreases in root:shoot ratio have been explained in terms of plant strategies to reduce costs to the overall plant carbon economy (Vartapetian & Jackson 1997), whereas increases have been associated with the production of adventitious roots (Parolin 2001). In our study different responses were observed across treatments, which suggests that waterlogging and soil fertility interact to promote varying responses. For example, in FW soils the three species that attained high root:shoot ratio (*P. colorata*, *C. foetidissima* and *L. scoparium*) also increased in relative growth rate (Fig. 1c): and this was correlated with presence of adventitious roots. In other cases reductions in root:shoot ratio promoted growth, for example in *N. solandri* and *W. racemosa* growing in FW but not in IW soils. Finally, we also found invariant root:shoot ratios, for example in *G. littoralis*, which developed adventitious roots under waterlogged conditions and achieved 100% survival across treatments (Table 3) Thus, some responses to waterlogging that are not reflected in allocation patterns and short-term growth may play a key role in species survival.

Growth rate was positively related to SRL for plants grown on FD soils (Fig. 3). These results are consistent with previous studies made on woody species. It was observed by Wright & Westoby (1999) for 33 woody species from sclerophyll vegetation in Australia, and by Huante, Rincon & Gavito (1992) for species associated with the understorey of tropical deciduous forest in Mexico. The most likely explanation is that higher SRL leads to a greater capacity to acquire nutrients per biomass invested in root construction (Comas & Eissenstat 2004). The positive relationship between growth rate and SRL has also been found to hold true under waterlogged conditions (Rubio, Casasola & Lavado 1995; Rubio *et al.* 1997). Exposure to waterlogging induces root aerenchyma, which lowers root biomass per unit length and hence gives rise to higher SRL (Loreti & Oosterheld 1996; Rubio *et al.* 1997). In our study, an increase in SRL under waterlogging was only observed for the two species from the oldest-terrace soils, and did not result in higher growth rate. It may be that increases in SRL only promote growth under waterlogged conditions if there are changes in root anatomy, which was not the case here.

SPECIES ASSOCIATED WITH RETROGRESSIVE SUCCESSION WERE NOT STRESS TOLERATORS IN THE CLASSIC SENSE

The two species common in the retrogressive stages of the Waitutu Chronosequence were tolerant of waterlogging, but were atypical of stress-tolerators (*sensu* Grime 1979) in their response to nutrients: they responded to soil fertility, achieved high growth rates and SRLs, and had relatively low allocation to roots. However, some species on the oldest soils do demonstrate classic stress-tolerance: the two conifers *Halocarpus biformis* and *Lepidothamnus intermedius* have long-lived leaves and very low photosynthetic rates (Gaxiola 2006). We would have liked to have included these species in our experiment but they very rarely produce seedlings. The success in poorly drained soils shown by *Nothofagus solandri* cannot be attributed to adventitious roots or aerenchyma, as the species produces neither of these (Sun *et al.* 1995). Instead, the superficiality of its rooting system – a shallow root plate, much like that of *Fagus sylvatica* trees in Europe (Wardle 1984) – appears to be important.

Responses of species associated with intermediate-age sites (P-depleted and relatively poorly drained soils) were more like those of the stress-tolerators envisaged by Grime (1977): low growth rates, high root:shoot ratios and inherent unresponsiveness to environmental heterogeneity (Lambers & Poorter 1992). The lack of response capacity may restrict their distribution to intermediate-age soils. For example, *M. umbellata* and the three conifers, which dominate the terrace forests, appear to be outcompeted by faster-growing angiosperms and dense ferns on the more recent nutrient-rich soils and disappear from communities on the older waterlogged soils (Coomes *et al.* 2005). Further work on root traits associated with survival under conditions of waterlogging and low nutrient availability will improve our understanding of the role of

these two factors in shaping these distinctive conifer-dominated species assemblages.

In conclusion, our investigation provides evidence that both P depletion and poor drainage combine to drive retrogressive succession through their impacts on species survival and growth capacity. The species studied here showed partly idiosyncratic responses to these two stresses, not always predictable by habitat association, and it was those from the intermediate-age rather than oldest-age sites that demonstrated classic characteristics of stress tolerance. Species growth and survival were explainable by observed modifications to root anatomy and biomass allocation patterns. These traits have a potentially important role in determining species distributions at different stages of the soil chronosequence where biogeochemical cycles reduce soil P availability and increase soil waterlogging.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Figure S1. (a) Allometric relationships between \log_{10} root biomass (g) and \log_{10} shoot biomass (g) and (b) \log_{10} root biomass (g) and \log_{10} leaf biomass (g), of 11 species grown in the following treatments: (●) fertile well drained, (○) fertile waterlogged, (▲) infertile well drained, and (△) infertile waterlogged. Lines represent significant type II regressions.

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