

EVOLUTIONARY ECOLOGY OF PLANT DEFENCES

Predicting root defence against herbivores during succession**Sergio Rasmann^{*1}, Taryn L. Bauerle², Katja Poveda^{3,4} and Rachel Vannette⁵**

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Summary

1. Root herbivores and pathogens interfere with basic below-ground plant function, and can thereby affect plant fitness and spatial and temporal patterns in natural plant communities. However, there has been little development of concepts and theories on below-ground plant defence, a deficit that is in contrast to the abundance of theorizing for above-ground plant parts.
2. A review of the past 10 years of research on below-ground plant–herbivore interactions has revealed that, similar to above-ground tissues, root defences can be expressed constitutively or induced upon herbivore attack, and can be classified into direct and indirect traits, tolerance, and escape. Indeed, it has been shown that roots tolerate herbivory by outgrowing or re-growing lost tissues, or resist it by producing secondary metabolites that are toxic to herbivores or attract natural enemies of herbivores.
3. We propose that, similar to above-ground plant–herbivore theories, the partition of abiotic and biotic factors over ecological succession can serve as the basis for predicting investment in defence strategies below-ground.
4. Investigation of herbivore pressure and root responses along primary and secondary successional gradients suggests that: (i) roots are often fast growing, thinner and softer in early compared to later succession. (ii) Insect and nematode herbivore pressure increases until mid-succession and later decreases. (iii) Mycorrhizal abundance increases with succession, and the composition of fungal species changes through succession, often shifting from arbuscular mycorrhizae to ecto-mycorrhizae.
5. Based on these findings, and on classical (above-ground) plant defence theory, we suggest the following set of testable hypotheses for below-ground plant defence: (i) During succession, early plants invest most of their resources in growth and less in defences (associated with a general lack of herbivores and pathogens, and with limited availability of resources in the system), therefore relying more on re-growth (tolerance) strategies. (ii) During mid-succession, a buildup of herbivore pressure facilitates replacement by plant species that exhibit greater direct and indirect defence strategies. (iii) Constitutive and inducible levels of defences may trade-off, and early successional plants should rely more on induction of defences after herbivore attack, whereas late successional plants will increasingly rely on constitutively produced levels of physical and chemical defence. (iv) Successional changes in microbial associations have consequences for root defence by improving plant nutrition and defence expression as well as directly competing for root space; however, toxic or impenetrable root defences may also limit association with root symbionts, and so may constrain the expression of root defence.

Key-words: allocation, below-ground, direct and indirect defences, mycorrhiza, nematodes, plant defence theory, soil insect herbivores, tolerance, trade-off

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Introduction

Plants along a successional gradient vary in their patterns of above-ground defence allocation and palatability to herbivores. Generally, early colonizing plants of disturbed habitats are characterized by a suite of traits which allows them to take advantage of rapid rates of resource supply (Mooney 1972; Grime 1977; Bazzaz 1979; Davidson 1993). Above-ground early successional plant strategies include rapid leaf turnover, comparatively little investment in defensive secondary compounds, and rapid re-growth when compensating for tissue loss (Bryant, Chapin & Klein 1983; Coley 1988). In contrast, late successional plant species have intrinsically slower growth rates, comparatively long-lived leaves (Grime 1977; Coley 1983), and tend to direct substantial amounts of carbon into physical defence (e.g. thorns) and highly toxic secondary metabolites or digestibility reducers (e.g. tannins) (Feeny 1976; Rhoades 1979; Coley, Bryant & Chapin 1985; Leps, Novotny & Basset 2001). It is therefore generally assumed that early to middle successional plants experience higher levels of above-ground herbivory (Edwards-Jones & Brown 1993). These observations prompted the formulation of fundamental hypotheses for the evolution and diversification of plant defence strategies (Ehrlich & Raven 1964; Odum 1969; McKey 1974; Cates & Orians 1975; Feeny 1976; Rhoades 1979; Coley, Bryant & Chapin 1985; Stamp 2003). In general, these hypotheses predict that above-ground plant defence strategies should represent the integrated outcome of abiotic resources such as available nutrient supply (Bryant, Chapin & Klein 1983; Coley, Bryant & Chapin 1985), biotic factors such as plant growth rate, herbivore pressure or competition (Hairston, Smith & Slobodkin 1960; Feeny 1976; Herms & Mattson 1992; Wardle & Barker 1997) along with evolutionary and genetic constraints (Ehrlich & Raven 1964).

Despite the fact that below-ground herbivores have a strong impact on plant fitness (Gange & Brown 1989; Reichman & Smith 1991; Prins, Nell & Klinkhamer 1992; Saner & Müller-Schärer 1994; Hunter 2001; Blossey & Hunt-Joshi 2003), and can shape successional patterns (Brown & Gange 1989, 1992; De Deyn *et al.* 2003), there remains a substantial

gap in our understanding of below-ground plant–herbivore interactions. Recent syntheses on below-ground responses to herbivores (Bezemer & Van Dam 2005; Erb *et al.* 2008; Kaplan *et al.* 2008; Rasmann & Agrawal 2008; Van Dam 2009; Van der Putten *et al.* 2009), question whether root responses to herbivory reflect above-ground responses, and affirm that roots can cope with below-ground herbivory by (i) Tolerance: organ re-growth through the utilization of stored reserves (Schmid, Miao & Bazzaz 1990; Van der Putten 2003); (ii) Direct resistance: the constitutive production or the increase after herbivore attack (i.e. induction) of a wide variety of toxic secondary compounds (Kaplan *et al.* 2008; Rasmann & Agrawal 2008; Van Dam 2009); (iii) Indirect resistance: attracting ‘bodyguards’ (predators of herbivores) to the site of wounding by the constitutive releasing or the induction after herbivore attack of volatile organic compounds into the soil (i.e. indirect defences) (Rasmann *et al.* 2005; Ali, Alborn & Stelinski 2010); and (iv) Escape/Avoidance: redirecting roots to enemy-free space (De Kroon & Mommer 2006) or store reserves in undamaged tissues (Van der Putten 2003) (see Table 1 for a classification of all putative below-ground plant defence strategies). While these patterns suggest a broad congruence of the types of above- and below-ground defences, there is still no predictive framework, based on soil conditions and plant responses, for when and where these different defences should be deployed in order to maximize plant fitness.

In this review, we aim to assess whether below-ground abiotic and biotic soil conditions predict plant defence strategies along successional gradients, similar to above-ground plant tissues. Based on classic work on above-ground plant defences, we start with the assumption that abiotic resources such as soil nutrient supply, and biotic factors such as root growth rate, herbivore pressure and symbiotic associations should shape plant defence response below-ground, within the genetic constraints imposed by the plant’s physiology and evolutionary history. We also start with the assumption that root defences should be closely tied to soil characteristics, which change dramatically over ecological succession. As a result, our predictive framework for root defence strategies takes succession explicitly into account, in addition to the

Table 1. Summary of root defence traits against soil herbivores and pathogens, and examples of possible mechanisms

Defence type	Example	Mechanisms
Tolerance	Regrowth	Reallocation of resources from other tissues
Direct defences	Overcompensation	Increase in plant’s primary metabolism after herbivore damage
	Chemical defences	High concentration of secondary metabolites at the site of wounding
	Structural defences	Toughness of tissue reduce herbivore ability to penetrate the roots
Indirect defences	Nutritional	N (C : N ratio)
	Tritrophic interactions	Recruitment of entomopathogenic nematodes by the emission of volatiles from the site of wounding
	Competitive exclusion	Arbuscular mycorrhizae outcompete phytophagous nematodes for space and resources.
Escape/avoidance	Phenological escape	Delayed growth
	Physiological escape	Storage of nutrients in less attacked tissue.
	Behavioural escape	Directional growth towards pathogen-free soil.

integrated effects of all the forces listed above (Odum 1969; Cates & Orians 1975; Van der Putten 2003; Agrawal & Fishbein 2006). We first reviewed the literature on plant succession (Table S1, Supporting information), and highlighted patterns of abiotic soil conditions (soil depth and soil nutrients), and biotic conditions [root biomass, morphology, growth rate and turnover, symbiotic associations (mycorrhizal associations) and below-ground herbivore load (insect and nematodes)]. These patterns are summarized in Figs 1 and 2. Based on these patterns we formulated specific predictions of plausible types of defensive strategies below-ground and how they should change over ecological succession. We specifically chose a variety of primary and secondary succes-

sion studies to increase the potential for generalizable predictions. Although we acknowledge that all soil organisms, including microbes such as bacteria and fungi, and micro- and meso- fauna such as soil arthropods and nematodes, may be important to plants and plant communities by both direct feeding and modifying the availability of resources (Wardle 2002), we chose to focus our attention on insect and nematode damage, as well as on plant–insect–mycorrhizal fungus interactions. Root herbivorous insects can be very abundant and species-rich (Whittaker 2003); and together, insects and root-feeding nematodes are considered the most ubiquitous root herbivores in natural and agricultural systems potentially consuming up to one half of primary production in

Fig. 1. Changes in soil depth, root traits, mycorrhizal association and below-ground herbivore load (insects and nematodes) during plant succession from bare soil until a temperate hardwood forest climax (data are in Table S1, Supporting information). Soil depth increases along the succession, as well as root mass, and root diameter. However, a decrease in root turnover, and root length is generally observed. Herbivore abundance constantly increases until mid-succession then gently decreases late in the succession. In many temperate ecosystems, mycorrhizal fungal abundance increases with succession and community composition changes both within mycorrhizal types (arbuscular mycorrhizae or AMF, and ecto-mycorrhizae or ECM) as well as an increase in ecto-mycorrhizal fungal diversity and abundance. Drawing by Alex Paya.

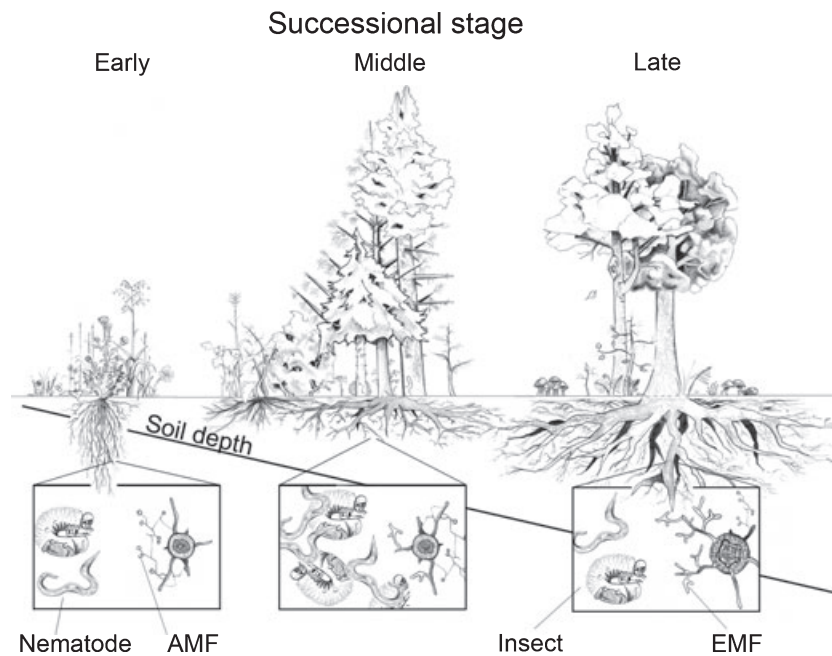
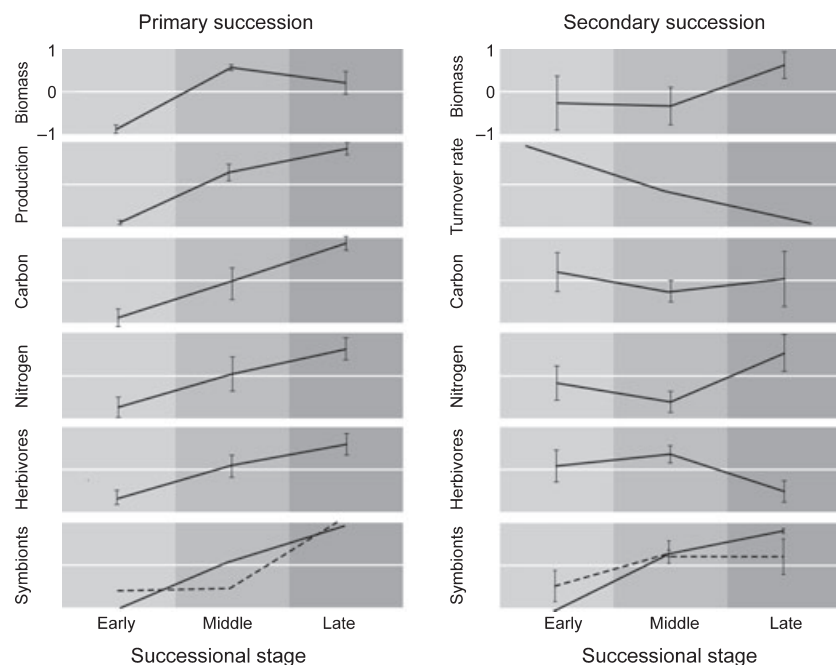


Fig. 2. Soil trait changes along primary and secondary successional gradients. Shown are root biomass, fine root production for primary and root turnover rate for secondary succession, soil carbon and soil nitrogen, soil herbivores (including both insects and nematodes), and symbiotic mycorrhizal associations [solid lines represent ecto-mycorrhizae (ECM), whereas dashed lines represent arbuscular mycorrhizae (AMF)]. Points of the lines represent averages (± 1 SE where more than one study is represented) of Z-score values calculated from different studies listed in Table S1, Supporting information. The zero line represents the average value across the full successional gradient and everything above or below this line represents the relative deviation from the mean.



some systems (Stanton 1988), and also being important drivers of ecological succession (De Deyn *et al.* 2003; Van der Putten 2003; Whittaker 2003). Because of their importance as agricultural pests, there has been substantial interest in identifying plant defences against nematodes and insects.

We additionally focused on mycorrhizal fungi because these form symbioses with at least 80% of all vascular plants (Wang & Qiu 2006), change in abundance and composition throughout succession (Johnson *et al.* 1991), and can substantially affect root nutrition, defence and morphology, thereby altering interactions with herbivores (Vannette & Hunter 2009). Association with mycorrhizal fungi can alter patterns of plant defence, tolerance and herbivore performance (Bennett, Alers-Garcia & Bever 2006; Hartley & Gange 2009) through multiple mechanisms, including increased nutrition, direct competition for root space or changes in gene expression related to defence (Borowicz 2001; De La Peña *et al.* 2006; Vannette & Hunter 2009). For example, mycorrhizal colonization of strawberry decreased the survival and biomass of root-feeding weevils and decreased the herbivores negative effects on plant biomass (Gange 2001). Similarly, mycorrhizal colonization of the dune grass *Ammophila arenaria* decreased the infection and reproduction of *Pratylenchus penetrans*, a migratory root-feeding nematode (De La Peña *et al.* 2006). Other studies have also found that mycorrhizae confer significant protection against root knot and cyst nematodes (Hol & Cook 2005; Siddiqui & Akhtar 2007; Deliopoulos, Haydock & Jones 2008; Zhang *et al.* 2008), although the mycorrhizal fungal colonists vary in their effects on nematode performance and plant response. Finally, we focus our attention on below-ground defences specifically, and not on the well-reviewed interactions with above-ground organisms (e.g. Bardgett & Wardle 2003; Bezemer & Van Dam 2005; Erb *et al.* 2008; Kaplan *et al.* 2008; Rasmann & Agrawal 2008; Erb *et al.* 2009; Van Dam 2009).

Dissecting the soil environment along plant successional gradients

Studies comprising a wide variety of plant successional patterns were chosen to encompass the full successional timeline, from either bare soil (primary succession) or from major resetting events (secondary succession) (Figs 1 and 2). Data were then categorized into three major successional stages (early, middle and late) and pooled by information on soil abiotic factors (soil depth, available N and C), and biotic factors (root biomass, root : shoot ratio, root morphology, root life span, turnover rate, root toughness, herbivore load and mycorrhization) (see Table S1, Supporting information). Data for root biomass, root turnover rates, available carbon and nitrogen, below-ground insect and nematode abundance and arbuscular and ecto-mycorrhizae amounts were transformed into Z-scores (i.e. for every variable, the difference between each datum and the mean across the three successional stages, divided by their standard deviation), which allowed comparisons among studies and types of measurements. Thus, Figure 2 represents the values of each variable

for each of the three successional stages as the average number of standard deviations from the mean value across the entire successional gradient.

ROOT ECO-PHYSIOLOGICAL TRAITS

Teasing apart differences in root traits between primary and secondary succession has remained a challenge due to the paucity of studies on primary succession or shifts in root development as a function of soil development. It did appear that nutrient-poor (e.g. early succession) sites favour long-lived roots with high nutrient reabsorption ability (Aerts 1997), perhaps in part because soil developmental patterns take longer in primary succession (Wardle *et al.* 2004). While there are few data, root traits across primary successional stages are clearly predicted to be linked to increased soil weathering and organic matter accumulation. Empirical studies that have estimated changes in root biomass during primary succession have found increased root production, turnover, biomass and a deeper root system with ecosystem age and subsequent soil development (Uselman, Qualls & Lilienfein 2007).

While soils of secondary succession sites can also be nutrient-poor, they are more developed when compared to primary successional soils (Bardgett 2005). Hence, in secondary succession soil resource acquisition is improved in plants with greater root biomass (Table S1, Supporting information), but also with different morphological characteristics, including increased root length and root surface area, two traits that improve resource foraging (Eissenstat 1991; Fitter & Stickland 1991). Empirical studies on secondary successional species have identified a suite of common root traits, including small root diameter, long root hairs, and low tissue mass density (TMD, see below) (Figs 1 and 2). In late secondary succession, root growth rate is lower (Tilman & Cowan 1989), and there is a higher allocation of resources towards the root system when compared to early secondary successional species (Gleeson & Tilman 1994) which contributes to higher densities of pathogens and herbivores (Darcyburtt & Blackshaw 1991). As secondary succession progresses a decrease in palatability and nutritional quality takes place. This may occur in part due to increased TMD, a proxy for 'root toughness' that possibly mediates, in part, a trade-off between resource acquisition and life span of the roots (Hummel *et al.* 2007). Additionally, contrary to deeper rooting depth trends found during primary succession, secondary successional rooting patterns become more shallow with time perhaps due to higher levels of nutrients near the soil surface (Brassard, Chen & Bergeron 2009), which in turn might contribute to a constant increase in herbivore levels until mid-successional communities (see later).

HERBIVORES

Interactions between plant roots, invertebrate herbivores and natural enemies of the herbivores have been shown to contribute to the relative abundance (Van der Putten, Van Dijk &

Peters 1993; Wardle & Barker 1997) and diversity (De Deyn *et al.* 2003) of plants throughout succession. Although we recognize that microbial pathogens can also be important drivers of succession (Van der Putten, Van Dijk & Peters 1993), we focus our attention on root-feeding nematodes and insects.

Along primary succession, below-ground herbivore load tends to steadily increase from early to late succession (Fig. 2). The highest herbivore loads in late primary succession are reported mainly in sand dunes, where the abundance and diversity of plant feeding nematodes increases as succession progresses (Wasilewska 1970, 1971; Zoon, Troelstra & Maas 1993; Goralczyk 1998). However, a study by Neutel *et al.* (2007) shows a decrease in herbivore density late in primary succession, perhaps because this study used late successional sites that were 50–100 years old, compared to 20 year old sites in earlier studies. This decrease in herbivore load late in primary succession is in accordance with data for secondary succession. Over the course of secondary succession the abundance of herbivores generally declines, but the pattern at mid-secondary succession varies on a case-by-case basis. In grassland secondary succession, the number of both root-feeding nematodes and beetle larvae increases until mid-succession and later decreases (De Deyn *et al.* 2003). In other cases there is a constant decrease in herbivore load with succession. For example the abundance of plant feeding nematodes decreased over a successional timeline after elimination of the fertilizer treatment in grasslands (Verschoor *et al.* 2001) or land abandonment in heathlands (Holtkamp *et al.* 2008). The latter study, in contrast, found a steady increase in total herbivore load after field abandonment (Holtkamp *et al.* 2008). Except for this one study we can suggest that in general herbivore load below-ground increases steadily to a certain point in mid-secondary succession, and then declines in later successional stages.

HERBIVORE SPECIALIZATION BELOW-GROUND

Until now it has been suggested that insect herbivores on roots facilitate the establishment of late successional plant species by feeding selectively on early, less defended, successional plant species (Brown & Gange 1992; De Deyn *et al.* 2003; Schadler *et al.* 2004). However, no study has addressed the mechanisms underlying this selective feeding. It could be argued that there is a certain degree of herbivore specificity causing selective feeding on early successional plants. Do herbivores become less and less specialized underground along succession? Feeny's (1976) plant apparency theory suggested that forest trees, with high levels of a broad range of digestibility reducers such as tannins or other polyphenols, might favour colonization by a more generalist suite of herbivores above-ground. Below-ground, it has been suggested that the increase in arthropod diversity found during mid-succession is the result of the gradual colonization by specialist herbivores (Mortimer, Van der Putten & Brown 1999). For example, root knot nematodes feed preferentially on dicots, which may in turn favour grasses (De Deyn *et al.* 2004). On the other hand, generalists such as wireworm larvae (Coleoptera:

Elateridae), or generalist plant-feeding nematodes may also expedite succession (De Deyn *et al.* 2003). Compelling evidence of below-ground herbivore specialization within a given plant community is still lacking, and given the limited dispersal ability of below-ground herbivores, specialization may be maladaptive. However, it should be noted that the ecology of individual herbivores seems to match the successional stages in which they live. In early successional patches, below-ground insect herbivores are represented by species with more widely dispersing adults and short-lived larval stages, while later successional stages have long-lived larval stages (Clements, Bentley & Nuttall 1987).

MYCORRHIZAL ASSOCIATIONS

Mycorrhizal associations, which can strongly impact plant–herbivore interactions (Bennett, Alers-Garcia & Bever 2006; Hartley & Gange 2009), change through successional time and can drive or follow changes in plant community composition and soil characteristics (Hart, Reader & Klironomos 2001). Early in primary succession or following major disturbance, mycorrhizal fungi are nearly absent in the soil, and as a result, initial colonists tend to be non-mycorrhizal (Allen *et al.* 1995; Cazares, Trappe & Jumpponen 2005). However, as fungal spores are dispersed, arbuscular mycorrhizal fungi (AMF) become the primary symbionts of mid-successional or grassland plant communities. With the encroachment of woody species and transition to temperate forest communities, ecto-mycorrhizal (ECM) fungi also increase in abundance (Uibopuu *et al.* 2009). Fungal associates of the 'climax' community vary among habitats (Smith & Read 2008): tropical forest trees and prairie plants predominately associate with AMF, late successional temperate forests also sustain AMF-dependent forbs in the understory, while conifers associate primarily with ECM (Uibopuu *et al.* 2009). While both mycorrhizal fungal types increase plant nutrition and growth, and can increase root life span (Atkinson *et al.* 2003; Guo *et al.* 2008), AMF and ECM may have different effects on root physiology, function and both symbioses are affected by and can alter insect herbivory (Bennett & Bever 2007; Gange 2007). Both mycorrhizal types convey nutrients to host plants (Smith & Read 2008); however, the morphology of these symbioses allows them to confer different benefits to plants with regard to below-ground defence. Arbuscular mycorrhizae may directly compete with root pathogens or herbivores for root space or resources (Borowicz 2001). ECM fungi, on the other hand, form a protective sheath around the exterior of the plant's finest roots, increasing root toughness and extending root life span up to 45% (Guo *et al.* 2008). While substantial evidence indicates that AMF often increase plant defence and decrease below-ground herbivore performance (Grandmaison *et al.* 1993; Peipp *et al.* 1997; Perner *et al.* 2008; Schliemann, Ammer & Strack 2008), the effects of ECM range from positive to negative, but are often weak or undetectable (Gehring & Whitham 1994; Manninen *et al.* 2000; Baum *et al.* 2009). Successional increases in fungal abundance, as well as shifts in the fungal

community among fungal species, are both likely to mediate changes in the expression of plant defences (Baum *et al.* 2009), as well as tolerance to herbivory (Bennett & Bever 2007; Garrido *et al.* 2010) and protection of plant roots (Bennett, Alers-Garcia & Bever 2006).

Predicting plant defence strategies along successional gradients

Roots of plants can deploy a variety of strategies to cope with soil-borne herbivores and pathogens, ranging from having the ability to regrow or escape the herbivore, producing toxic secondary metabolites or physical defence barriers and signalling enemies of the herbivores their presence near the roots. Here, we argue that variation in below-ground defensive traits (Van Dam & Vrieling 1994; Nestby & Heiberg 1995; Kollner *et al.* 2008; Kabouw *et al.* 2010; Rasmann *et al.* 2010) and the prevalence of a particular defence strategy is the result of a combination of succession-specific distinct abiotic and biotic conditions, plant physiological traits and evolutionary constraints.

Specifically, we propose that: (i) Tolerance as a root defence strategy will decrease in relative importance along succession. (ii) Direct physical and chemical defences will increase in frequency with succession in a linear fashion. (iii) Indirect defences should be most important and prevalent in middle successional stages. (iv) Inducible defences should be favoured at the beginning of succession but then decrease, with later stages being characterized by higher levels of constitutive defence. (v) Increasing mycorrhizal abundance later in succession will facilitate the expression of constitutive defence, as well as the decline in inducible defences and tolerance along succession, and will also offer specific protection against nematodes.

ESCAPE AND TOLERANCE STRATEGIES

Above-ground, plants may avoid their herbivores or pathogens through phenological escape, whereby they expose vulnerable tissues (leaves, flowers) early (or late) in the growing season, or exhibit spatiotemporal unpredictability (Van der Meijden, Wijn & Verkaar 1988). Below-ground, roots might also be able to reduce herbivory by delaying their phenology, such as growing in late autumn, winter or early spring (Bauerle *et al.* 2007). However, we agree with Van der Putten (2003) that complete escape is only possible when plants reach new territories where no similar herbivores are present, which may not be possible in a successional sequence.

Plants have been shown to tolerate severe tissue loss above-ground (i.e. herbivore damaged plants have the same fitness than undamaged plants), and compensation is the degree of tolerance exhibited by plants (Strauss & Agrawal 1999). In some cases, plants can overcompensate for the damage and produce more biomass or fitness units than they would if undamaged (Lennartsson, Nilsson & Tuomi 1998; Poveda, Kessler & Gomez-Jimenez 2010). Literature on plant compensatory mechanisms after above-ground herbivory sug-

gests that plants should be under natural selection to compensate if: (i) herbivory is continuously very strong; (ii) there is only one bout of herbivory; (iii) the risk of herbivory occurs before flowering; (iv) most of the resource acquisition occurs before herbivory; and (v) the abiotic conditions such as nutrient availability are not limiting (Maschinski & Whitham 1989; Vail 1992; Strauss & Agrawal 1999; Agrawal 2000). Under these conditions, the optimal plant strategy would be to withhold reproductive resources against the possibility that an initial investment would be consumed. As above-ground, where plants have been shown to tolerate severe tissue loss (Lennartsson, Nilsson & Tuomi 1998); below-ground, under controlled experimental conditions, a large fraction of plant root systems can often be removed without negatively affecting plant biomass production (Van der Meijden, De Boer & Van der Veen-Van Wijk 2000). It has been shown that plants are able to compensate for damage caused by below-ground herbivory by regrowing the lost tissue (Quinn & Hall 1992; Newingham, Callaway & Bassirrad 2007) or even producing more tissue than the one eaten by herbivores (overcompensation) (Muller 1989). We predict that in combination with other stresses such as low nutrients or drought, the loss of below-ground tissue is much more difficult to tolerate than the loss of foliar tissue (Andersen 1987) and for this reason we hypothesize that tolerance strategies below-ground are favoured under a different set of conditions than above-ground.

We know little about how tolerance strategies below-ground vary along a successional gradient. It has been shown in potatoes that plants can compensate for damage to tubers in cases of low, but not high herbivory (Poveda, Kessler & Gomez-Jimenez 2010). Additionally, increasing mycorrhizal colonization (as found in later successional stages) decreases plant tolerance of simulated foliar herbivory in *Datura stramonium* (Garrido *et al.* 2010). Similarly, mycorrhizal colonization of *Agrostis capillaris*, a perennial grass, decreases its tolerance of below-ground herbivory by *Tipula paludosa* larvae (Currie, Murray & Gange 2006). Fungal species identity seems to determine the effect of mycorrhizal colonization on plant tolerance to damage (Bennett & Bever 2007); and these effects may also be determined by fungal life-history and successional status (Hart, Reader & Klironomos 2001). However, mycorrhizal fungi also slightly increase the compensation of mycotrophic grasses following insect herbivory (Kula, Hartnett & Wilson 2005). Based on this limited evidence, we hypothesize that tolerance is a defence strategy that should mainly be used by plants subjected to low herbivore pressure and those not heavily colonized by mycorrhizal fungi. Given that herbivore pressure and mycorrhizal colonization in middle and late succession are generally high (Fig. 2), we predict that tolerance mechanisms should be more important for early successional species than later in succession (Fig. 3). However, it should be noted that tolerance and resistance are not necessarily strict alternative strategies (Simms & Triplett 1994), and that intermediate levels of resistance and tolerance may be favoured by natural selection under some conditions (Nunez-Farfan, Fornoni & Valverde 2007). This hypothesis

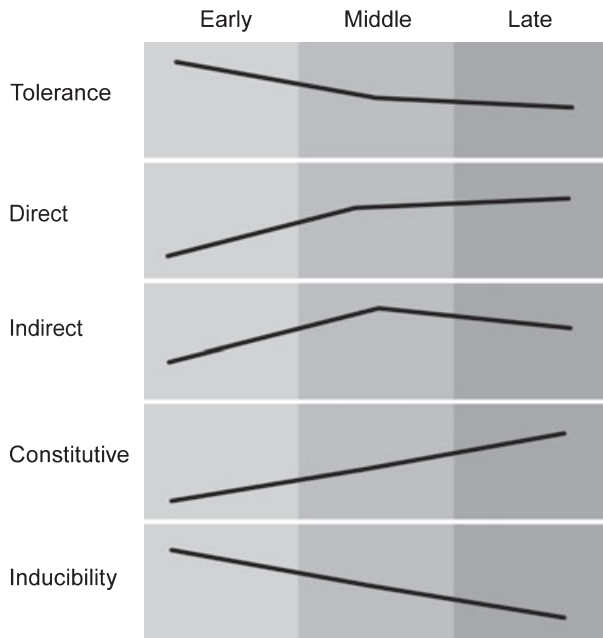


Fig. 3. Diagram showing prediction of how plants allocate in different root defence strategies through succession. Shown in order are tolerance, direct defences, indirect defences, constitutive deployment of defence and the ability to induce defences. See text for explanation.

needs to be tested by, for instance, measuring tolerance and resistance traits on roots damaged by generalist and specialist herbivores on plants from the different successional stages.

DIRECT DEFENCES

If trade-offs between growth rate and defence can be generalized from leaves to the whole plant, slower-growing plants should have better-defended roots (Grime 1977; Coley, Bryant & Chapin 1985; Endara & Coley 2011). Indeed, direct observation of root infestation of both fast- and slow-growing root systems has shown that fast-growing plants produce more root tips and are subject to greater herbivory per individual root (Bauerle *et al.* 2007). This would lead us to predict that late successional plants should be more defended than early successional plants (Fig. 3). Although it has been shown that late successional plants are better defended against above-ground herbivory than early successional plants (Cates & Orians 1975; Coley, Bryant & Chapin 1985; Poorter *et al.* 2004), empirical evidence for a causal relationship between changes in defences below-ground and herbivore pressure is still non-existent. Indeed, structural defences which provide a direct physical barrier for herbivores (Johnson *et al.* 2010) are higher in mid- late-succession roots (Bazzaz 1979), where plants experience higher levels of herbivory (Figs 1 and 2). Alternatively, successional changes in soil communities may also contribute to changes in defence along successional gradients, and the presence of mycorrhizal fungi often increases the concentration of plant defence compounds in roots. Mycorrhizal fungi presence increases the concentration of triterpenes, isoflavonoids, phenolics, hydroxycinnamic acid

amides and flavonol glycosides in *Cucumis sativus*, *Medicago truncatula*, *Allium porrum*, *Hordeum vulgare* and *Allium cepa* respectively (Grandmaison *et al.* 1993; Peipp *et al.* 1997; Akiyama & Hayashi 2002; Perner *et al.* 2008; Schliemann, Ammer & Strack 2008). In addition, increasing abundance of mycorrhizal fungi can also correspond to changes in above-ground plant defences in *Asclepias syriaca* (Vannette & Hunter 2011) and like fungal effects on tolerance, the effect of fungal abundance on plant defence is fungal species-specific and may correspond to successional status.

In the same context, because different orders of roots vary in their function and longevity (Eissenstat & Yanai 1997), we predict root order to correlate with root defence patterns as well (Wells & Eissenstat 2001). For example, as a root only 1 mm in diameter may have several orders of finer roots depending on it for transport, there is greater value in defending this root than the finer-order roots. A study comparing five *Brassica* species consistently showed that primary roots had the highest levels of glucosinolates (Van Dam, Tytgat & Kirkegaard 2009). Similarly, main roots of *Senecio jacobaea* plants contained higher levels of pyrrolizidine alkaloids than the fine roots, independent of the nutrient concentration on which the plants were grown (Hol, Vrieling & Van Veen 2003). Moreover, the coarse roots of perennial trees are more lignified than roots of annual plants, or of finer, lower order roots (Pregitzer *et al.* 1997), potentially providing a stronger physical barrier to herbivores and pathogens alike, as suggested for leaves (Feeny 1970; Haukioja 2003).

Variation in leaf life span has been a strong predictor of variation in foliar defence (Coley, Bryant & Chapin 1985; Endara & Coley, 2011). However, plants may have less control over root life span than they do over leaf life span. Roots observed in minirhizotrons are commonly seen to disappear rather than senesce and decompose in place (Johnson *et al.* 2001). Insecticide and fungicide applied to soil extended the life span of roots by 46–125 days in peach, and more than 500 days in sugar maple (Wells, Glenn & Eissenstat 2002). Associations with both arbuscular and ECM fungi also tend to increase root life span (Atkinson *et al.* 2003; Guo *et al.* 2008). If herbivores, pathogens, and soil mutualists control the death of roots to a greater extent than intrinsic physiology, then theories of root defence may be able to place less emphasis on root life span.

INDIRECT DEFENCES

It is generally accepted that evolutionary processes and resulting adaptation in plant–herbivore interactions cannot be fully understood without consideration of the antagonists of herbivores in a multitrophic framework (Price *et al.* 1980). Above-ground, damaged leaves or stems are able to produce nectar secretions or volatile organic compounds to attract enemies of the herbivores (Heil 2008). Although still meager, current evidence on tritrophic interactions below-ground suggests that below-ground indirect defence may be ubiquitous. Predatory nematodes, mites or parasitoids are attracted to insect damaged roots of both annual and cultivated grasses and

herbs (Boff, Van Tol & Smits 2002; Neveu *et al.* 2002; Aratchige, Lesna & Sabelis 2004; Rasmann *et al.* 2005; Rasmann & Turlings 2008), old-field forbs such as the common milkweed *Asclepias syriaca* (Rasmann *et al.* 2010), conifers such as *Thuja* spp. (Van Tol *et al.* 2001) or perennial cultivars such as citrus plants (Ali, Alborn & Stelinski 2010). Also, specialist root-feeding nematodes of coastal fore-dune grass *Ammophila arenaria* (marram grass) are able to distinguish and avoid roots of the host plant when infested with harmful micro-organisms (Piskiewicz *et al.* 2009).

Production of signalling volatile organic compounds in the soil may however be manipulated by other soil inhabitants. For example, mycorrhizal fungi have been found to suppress the induction of defences against above-ground herbivores (Laird & Addicott 2007; Bennett, Bever & Bowers 2009), and may similarly suppress induction of responses below-ground. Mycorrhization can also prime plants to more quickly respond to insect attack (Pozo & Azcon-Aguilar 2007). We here suggest that although the expression of indirect defences may be limited by mycorrhizal fungi, what drives plants to invest in the production of volatile organic compounds below-ground is highly driven by the presence or absence of higher trophic level organisms in the soil (Fig. 3). Empirical evidence for increased predator presence below-ground along a succession has been reported (Wasilewska 1970; Koehler 2000), while a decrease of predators and pathogens has not yet been found (Goralczyk 1998; Wasilewska 2006; Chauvat *et al.* 2009). For this reason, we would expect indirect defences to be more important in late successional stages than in early succession.

The suggestion that both direct and indirect defences should predominate in late succession may seem paradoxical given the widespread assumption that these two defence strategies represent a supposed trade-off as predicted based on resource allocation theory (Strauss *et al.* 2002; Agrawal, Conner & Rasmann 2010). However, trade-offs in defence are in fact rarely found and are often idiosyncratic (Strauss & Agrawal 1999; Koricheva, Nykanen & Gianoli 2004). Additionally there is now indication that both direct and indirect defences may provide redundancy or 'reinforcement', enhancing protection against below-ground herbivores (Rasmann *et al.* 2010).

CONSTITUTIVE VS. INDUCIBLE DEFENCES

The evolution of inducible, direct and indirect defences is predicted under variable, but costly herbivore damage, when the cost of constitutive expression is not adaptive when weighed against the probability of attack (see Karban, 2011). We found that herbivory is less consistent early in succession, but becomes more predictable in late succession (Table S1, Supporting information). These results, in conjunction with optimal allocation theory (McKey 1974; Zangerl & Rutledge 1996) lead us to predict that early succession roots should be characterized by lower constitutive defences, but higher inducibility, with the opposite pattern late in the succession. Contrary to different strategies such as direct vs. indirect defences, the differential deployment of the same defence (i.e.

maintaining high constitutive levels or mounting the defence only after attack) uses resources directly from the same biosynthetic pathway, which is likely to result in a negative correlation between constitutive production of defences and their inducibility (Fig. 3). Accordingly, the first study to examine the relationship between constitutive and induced deployment of both direct and indirect root defences showed strong negative genetic correlations between constitutive allocation to root cardenolides and their inducibility (defined as the absolute increase in cardenolides following damage), and between constitutive allocation and inducibility of total root volatiles across 11 genotypes of *Asclepias syriaca* (Rasmann *et al.* 2010).

Although constitutive and induced defences may trade-off, the expression of these defences may also be limited by plant dependence on soil mutualists. For example, theory suggests that root fungi can suffer from non-target effects of anti-herbivore secondary metabolites, suggesting additional below-ground ecological costs to defence expression (Strauss *et al.* 2002). *Plantago lanceolata* clones high in iridoid glycosides displayed reduced mycorrhizal colonization in comparison to clones containing less iridoid glycoside (De Deyn *et al.* 2009). In addition, increased root defences (phenolics and lignin) led to a decrease in the amount of ECM fungi in roots of paper birch, *Betula papyrifera* (Kleczewski, Herms & Bonello 2010). It is possible that this ecological cost would oppose selection for the production and deployment of toxic secondary metabolites in roots.

OUTLOOK

The past decade has seen a steady increase in the number of studies on below-ground plant defences against herbivores, and we are now at a point where studies of below-ground defence against herbivores require testable hypotheses and predictions. We advocate an integrative approach to shorten our gap in understanding, and particularly, we urge integration of knowledge of plant primary and secondary metabolism, along with studies of the community structure of plants and herbivores along natural ecological gradients and finally with micro- and macro-evolutionary forces shaping plant traits. The framework we present here provides some specific hypotheses and predictions, and lays the groundwork for future progress in this field. However, we note three major gaps in the literature that need addressing in conjunction with these theoretical considerations: first, we need empirical, systematic measurements of herbivore abundance and diversity in the soils of different plant communities and ecosystems. Secondly, we need data on the relative fitness impact of soil herbivores on plants (preferably in comparison to above-ground herbivores), along with measurement of defensive and other ecological traits. Thirdly, we require basic estimates of the heritability of below-ground defences, and of genetic correlations between below-ground and above-ground plant defence deployment, and direct and indirect defence strategies. We are hopeful that the next 10 years will yield data on these and the other deficits in our knowledge of below-ground

trophic interactions and the conjunction of soil ecologist, ecophysiologicals, chemical ecologists and evolutionary biologist will lead to a synthetic understanding of plant defence as a whole.

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Supporting information

Additional supporting information may be found in the online version of this article.

Table S1. Analysis of the influence of different successional stages (early, middle and late) on plant-, abiotic- and biotic- traits.

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