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PLANT ANIMAL INTERACTIONS

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The resource economics of chemical and structural defenses across nitrogen supply gradients

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Abstract In order to better understand the role of nutrient supplies in determining the prevalence of plant defense types, we investigated the theoretical relationships between ecosystem N supply and the net C gain of shoots that were undefended or defended in one of three ways: (1) by N-free chemical compounds, (2) by N-containing chemical compounds, or (3) by structural defenses. By extending economic models of shoot resource balance to include the relative value of C and N, depreciation, and amortization, we were able to show that the relative net C gain of the three defense types were similar to changes in their generally understood abundance along an N supply gradient. At low N supply, the additional C acquired when investing C in defense is much higher than investing N in defenses. Only at high N supply is it better to invest large quantities of N in defense rather than additional photosynthesis. In a sensitivity analysis, net C gain of shoots was most sensitive to factors that affect the relative value of C and N and the rate of herbivory. Although there is support for the relative value of C and N influencing defense strategies, more research is necessary to understand why tannins are not more prevalent at high N supply and why moderate amounts of N-based defenses are not used at low N supply.

Keywords Carbon-nutrient balance · Spinescence · Herbivory · Resource economics

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Introduction

Herbivory is a major constraint on plant growth (Grubb 1992). Not only is the removal of biomass costly, but plant defenses also use resources that could be used for more productive purposes (Coley 1986, but see Koricheva 2002). For defenses to be effective against herbivory they must reduce the loss of resources from herbivory more than the amount required for the defense. Understanding the determinants of the effectiveness of plant defenses requires quantifying not only the benefits of a defense (reduction in resource loss by herbivory), but also the costs of a defense (resources allocated to defense) and how the costs and benefits are modulated by resource availability (Berenbaum 1995).

There are two general classes of defenses: chemical and structural. Chemical defenses are compounds stored in tissues that affect either the digestion of biomass or have toxic effects after being absorbed after ingestion. Chemical defenses are further differentiated by whether they contain N (e.g., alkaloids) or are N-free (e.g., tannins, phenols). We recognize that the costs of synthesis and storage of some N-free defenses require investment of N to produce the compounds (Berenbaum 1995), but ignore this potential variation for now. As with most N-free chemical defenses, condensed tannins (the case we examine here) are more prevalent at low N supply than at high N supply. Tannins precipitate protein and lower the digestibility of the biomass. For leaves with a high ratio of tannin to protein, there is no net N gain during digestion, as tannins bind to proteins in the digestive tract and render them

indigestible. Tannin concentrations in leaves can be as high as 20% (Swain 1979), but it is uncertain if there is a maximum content of tannin in leaves and therefore a maximum N concentration at which a given tannin:protein ratio can be maintained.

N-based defensive compounds generally are directly toxic to herbivores rather than affecting the digestion of biomass. They are usually stored in lower concentrations in mature leaves [often <1% of dry leaf mass (Bazzaz et al. 1987)], compared with much higher levels in buds and reproductive tissues (Gleadow and Woodrow 2000). Though there are many types of N-containing defensive chemicals, we analyze the costs and benefits of a hypothetical cyanogenic glycoside here. When tissues are damaged, cyanogenic glycosides come into contact with enzymes that release cyanide, which then binds to the electron transport system of herbivores and impairs metabolism. Cyanogenic glycosides are present in an estimated 11% of all plant species, distributed among all major plant taxa (Jones 1988).

Structural defenses include prickles, thorns, and spines that vary in size, robustness, and shape (Janzen and Martin 1981). Spines are produced on branches adjacent to leaves and serve to reduce the bite size of mammals browsing on leaves and therefore limit offtake (Gowda 1997). Flightless birds (ratites) also browse on plants (Atkinson and Greenwood 1989; Williams 1993) and the "wire-plant" strategy (W. Bond et al., unpublished data) works analogously to spines in reducing feeding rates. Instead of spines, which are useless against the hard-beaked birds, wire-plants have strong, elastic branches with small, widely spaced leaves and wide branching angle. The wire-plant strategy reduces the feeding rates of ratites by increasing the difficulty of snapping branches by pulling, reducing the leaf biomass that can be ingested per bite, and making the branches difficult to manipulate and swallow. Both spines and wire-plant traits are similar in that they primarily increase the C invested in branches for defensive purposes.

The prevalence of structural and chemical defenses depends on ecosystem nutrient supply. Plants from strongly N-limited ecosystems are generally defended by tannins, whereas N-based and structural defenses become more abundant with increases in N supply (McKey 1979; Gartlan et al. 1980). For example, in the savannas of southern Africa, infertile miombo woodlands and savannas on soils derived from highly weathered granites have trees whose leaves are defended by tannins, while on nearby savannas on higher-nutrient soils such as shales and young volcanic soils, plants are defended by spines.

Independent of the mechanisms by which defense levels respond to variation in resource supplies, it has been hypothesized that the changes in relative abundance of defense strategies with increasing N supply are associated with the relative costs and benefits of the different defenses (carbon-nutrient-balance hypothesis, Bryant et al. 1983; resource availability hypothesis, Coley et al. 1985). Mechanisms aside, the decrease in C-based chemical defenses with increasing fertility is hypothesized to be associated with the relative increase in the costs of acquiring C relative to N as N supplies increase. That not withstanding, attempts to calculate the resource costs and benefits of plant defenses have not included the relative value of the two resources (Bloom et al. 1985; Bazzaz et al. 1987). Generally, defense costs have been calculated based solely on the energy cost of the synthesis and maintenance of the defense, ignoring most of the cost of the N, i.e., the amount of resources spent on its acquisition. This can be sizable considering more than half of an N-limited plant's C budget can be allocated belowground.

The lack of the incorporation of the relative value of C and N may explain why previous analyses have contradicted the prediction that along a gradient of increasing N supply, C-based defenses should become more expensive and N-based defenses less expensive. For example, Skogsmyr and Fagerström (1992) calculated the cost of the C and energy directly associated with production and maintenance of nicotine, an N-containing defensive compound. They found that nicotine should be cheap even at low N availability, potentially a result of not incorporating the high cost of N acquisition into the cost of the defense.

Determining the costs and benefits of biomass and allocation of resources to purposes such as defense has been limited by the inability to calculate the cost of multiple resources in a common currency. If a unit of biomass or a defensive compound contains both C and N, the costs of both need to be expressed in a common currency in order to compare the costs of allocation strategies that differ in their relative amounts of C and N. For example, without being able to express the value of C and N in a common currency, there is no way of knowing if a defensive strategy that uses 45 mg C and 2 mg N is more expensive than one that uses 60 mg C and 1 mg N.

The relative value of C and N can be derived from the exchange rates of the two resources at the whole-plant or stand level (Bloom et al. 1985). In human bartering-systems, the relative value of a commodity is assessed on the empirical exchange rates between parties. For example, if on average three loaves of bread are exchanged for two pounds of butter, the relative value of one pound of butter is 1.5 loaves of bread. Analogously for plants, the relative value of two resources can be inferred from the exchange rates, i.e., how much C is spent by a plant relative to how much N it acquires and vice versa. Quantifying these exchange rates is best assessed at the whole-plant level (see Poorter 1989), since, for example, the costs of roots acquiring N can not be separated from the costs of leaves acquiring reduced C, which is then used to support the roots. If at the whole-plant or stand level, x g C is acquired per y g of N lost, the relative value of N in terms of C is x / y. Using this exchange rate, the cost of N in leaves can be expressed in terms of C. Assuming approximate steady-state conditions, acquisition and loss should be nearly equivalent and can be substituted for one another. It is important to note that both resources need to be co-limiting to growth for their relative value to be calculated. If a resource is not

limiting, then it theoretically has no value since additional units of the resource that are acquired will not lead to the acquisition of more resources.

In addition to incorporating the relative value of C and N, understanding the resource economics of structural defenses requires that cost-benefit calculations be adjusted to reflect the difference in longevity of the structural defenses of branches and the leaves they protect. Since spines and branches (of wire plants) provide defense over a period longer than the longevity of a leaf, the costs of producing the defense needs to be amortized over the period over which defense is active. For example, the spines of acacias are associated with leaves that are produced on short shoots. These spines will often protect leaves for multiple years and the one-time cost of producing spines needs to be spread out over the number of years for which the spine protects leaves. For wire plants, we have estimated that a given short shoot can be active for up to 10 years (W. Lee, personal observation).

Our first goal is to compare the relative economics of C-based, N-based, and structural defenses along an N-supply gradient via a cost-benefit analysis that incorporates the relative value of C and N explicitly in a manner that would be applicable for both genotypic and phenotypic variation in responses to variation in N supply. We test whether these cost-benefit analyses support predictions from carbon-nutrient-balance hypotheses and explain the prevalence of defense types across an N-supply gradient. The costs and benefits of the defense strategy are analyzed with and without herbivory. We hypothesize that differences among defenses in net C gain will be correlated with the relative prevalence of the defense type along an N-supply gradient. At low N supply, C-based defenses should have the highest net C gain, while at high N supply, N-based and structural defenses should have the highest net C gain. We base our calculations on a hypothetical stand of open-grown, deciduous woody plants that would be short enough to have all their leaves browsed by ground-based mammals. Along an N-supply gradient, we analyze the costs of three defenses (N-free tannins, N-containing cyanogenic glycosides, and spines) in the absence of herbivory. We then analyze the costs and benefits of each defense in the presence of herbivore pressure.

Our second goal is to understand the sensitivity of net C gain of a unit of shoot to variation in constituent parameters like leaf N concentration and the fraction of leaves that are eaten. For this purpose, we run a sensitivity analysis on the calculations of the costs and benefits of defense strategies to see which factors are the most important in determining the costs and benefits of a defense strategy, the influence of compound turnover rates on net C gain, and the benefits of reusing structural defenses over multiple years.

Materials and methods

Model development

We calculate the resource losses and gains of a standard mass of leaf (1 g), the woody branch biomass that is produced along with the leaf biomass, and any resources used to defend the shoot (leaves and adjacent branch) along an N-supply gradient for three different defense strategies. We investigate the costs and benefits of the defenses with and without herbivory of leaves.

In the model, leaves and branches are produced at the beginning of the growing season, leaves have a probability of being eaten, and then the leaves are senesced if they live to the end of the growing season. Costs associated with a shoot during the growing season are equivalent to its costs of construction and maintenance, while the benefits are equivalent to the C gain of the leaf (Gulmon and Mooney 1986) and the resources that are resorbed during senescence. Net benefits are equivalent to the difference between the benefits and the costs and are calculated in units of C. We assume the amount of resources used in support of the shoot biomass (all other plant resource allocation) does not change with differences in defense strategies and there are no additional costs with the production of defenses beyond the costs of the resources used in the target leaf and branch biomass. Therefore, these support costs are not included in our calculations. The equations incorporated in our model are listed in Appendix 1.

Benefits

The gross benefits of the shoot (Eq. 1) include the total amount of C photosynthesized less respiration during the longevity of a leaf and the resources remobilized from the leaf at senescence. We only evaluate the economics of shoot biomass produced at the beginning of the growing season and do not calculate the economics of the biomass that may be produced as a replacement to removed biomass. Annual leaf C gain is equal to the C gained per day multiplied by the average longevity of the leaf (Eq. 2). Daily C gain is the difference between photosynthesis (Photo) (12 h day^{-1}) and respiration (Resp) (24 h day^{-1}) (Eq. 3). Photosynthetic rates are calculated as a linear function of the N concentration of the leaf using a standard equation [Eq. 4 (Aber et al. 1996)]. We assume there is no shading of leaves, which simplifies the calculations, yet is reasonable for open-grown trees. N concentration of a leaf (Eq. 5) increases logarithmically with N supply (Nsup) based on Reich et al. (2001) (see below).

Respiration of the shoot (Eq. 6) increases linearly with the N content of the shoot (Reich et al. 1998). Shoot N content is equivalent to the sum of leaf and branch N, and we assume no difference in the relationship between respiration and leaf N or branch N. The N content of the branch (Eq. 7) is equal to the N concentration of branch wood (0.2%, Table 1) multiplied by the C content of the branch (Eq. 8), which is the sum of the C in the branch allocated for structure and the C allocated for defense. Defense C allocation depends on the defense strategy (Table 1, see below for descriptions of defenses). The N content in the leaf (Eq. 9) is equal to the N concentration of the leaf multiplied by the C structural content of the leaf plus any N that is allocated for defense.

The average longevity of a leaf (Eq. 10) is the weighted average of the longevity of eaten leaves and uneaten leaves. This is dependent on what proportion (Frac) of the shoot and leaf is eaten, the average age at which the leaves are eaten (set at 90 days), and the maximum longevity of the leaf (set at 180 days).

At the end of the season, any N that is remobilized is considered a benefit. No structural C is assumed to be resorbed from leaves and only a proportion of the N in leaves is remobilized. No resources are remobilized from branches. The average salvage value of the leaves (Eq. 11) is dependent on the proportion of the shoot that is browsed on by herbivores (see below) and the proportion of the leaves that are eaten per unit shoot browsed, the value of the N in the leaf in terms of C (Eq. 12) and the resorption efficiency of the plant

Table 1 Values of constants used in calculations of net C gain for leaves. *Def* Defense, *Photo* photosynthesis, *Resp* respiration

Parameter	Units	Value	Source
BranchDefLong	Years	3	
b, NLeaf	g N g^{-1} $Nyear^{-1}$	−0.0187	
b, Cgain	g C g^{-1} $Nyear^{-1}$	−43.9	
b, Photo	g C day^{-1}	0.141	Aber et al. (1996)
b, Resp	g C g^{-1} N day^{-1}	−0.00062	Reich et al. (1998)
CbranchDefense	g C g^{-1} leaf	0 [a]	
CBranchStructure	g C g^{-1} leaf	0.45	
CLeafDefense	g C g^{-1} leaf	0 [b]	
CLeafStructure	g C g^{-1} leaf	0.45	
GrowthResp	g C g^{-1} C	0.5	
k_{Photo}		0.76	Aber et al. (1996)
LeafAgeEaten	Days	90	
LongevityLeafMax	Days	180	
m_{Cgain}	g C g^{-1} $Nyear^{-1}$	913.2	
m_{NLeaf}	g N g^{-1} $Nyear^{-1}$	0.5062	
m_{Photo}	g C g^{-1} N day^{-1}	7.24	Aber et al. (1996)
m_{Resp}	g C g^{-1} N day^{-1}	1.28	Reich et al. (1998)
NBranch	g N g^{-1} C	0.002	
NLeafDefense	g N	0 [c]	
Nsupply	g N $year^{-1}$	3–30	
ReplacementLeaf	Days	14	
Resorption		0.5	

[a]CbranchDefense: Tannin (%NBranch×25), Spine (0.45)
[b]CleafDefense: Tannin (NLeaf×25), N (NLeafDefense*20)
[c]NLeafDefense: N(0.02)

(Table 1). We assume that N in the leaf allocated for defense is resorbed at the same rate as the rest of the N in the leaf.

The value of N in terms of C (Eq. 13) is equivalent to the amount of C gained at the stand level by a typical plant or stand divided by the N-supply ratio. Although these actual exchange values are going to differ among species and ecosystems, we use a generic relationship that allows us to examine the consequences for changes in exchange rates across the N supply gradient. Gross photosynthesis for determining these values was calculated using the PnET-Day model using algorithms and environmental input data described by Aber et al. (1996). PnET-Day is based on a multi-layered forest canopy where maximum leaf-level photosynthesis is determined as a linear function of leaf N concentrations. Actual rates of photosynthesis are determined according to the availability of solar radiation (which declines with canopy depth), soil moisture, temperature, and vapor pressure deficit. Leaf area index (LAI) is not an explicit input, but is determined from the combination of canopy biomass and specific leaf area. PnET-day serves as the core module of PnET-II and PnET-CN ecosystem models, which include additional processes such as biomass accumulation and N cycling.

Because PnET-Day is a canopy model that lacks N cycling and N mineralization calculations, we applied the model using foliar N and LAI values that represent canopy traits along broad gradients in N availability. We used foliar N concentrations of 1.1–2.8%, which correspond to values that occur in temperate forests at N mineralization rates ranging from approximately 20 to 160 kg N ha^{-1} $year^{-1}$ (after Ollinger et al. 2002 and Reich et al. 2001). Inputs for canopy mass varied from 120 to 500 g m^{-2}, which, for broad-leaved tree species, correspond to LAI values of approximately 1.5–6.

LAI was calculated as a linear function of N mineralization (LAI=0.0228×Nsupply+1.7545) and leaf N concentration a logarithmic function of N supply [Leaf%N=0.5062×ln(Nsupply) −0.0187]. Gross photosynthesis was calculated in Pnet-Day for N supplies from 3 to 30 g N m^{-2} $year^{-1}$. The final relationship between stand-level photosynthesis and N supply was a logarithmic function of N supply (Eq. 14, Fig. 1).

Biomass costs

The initial costs of the leaves and the branches (Eq. 15) are equal to the costs of the C and N in the leaves (Eq. 16) and the costs of the C and N in the branch (Eq. 17). The cost of the C in the leaf (Eq. 18) is equal to the amount of C used for defense, structure, and growth respiration (Eq. 19). Growth respiration (Eq. 20) is calculated as a constant fraction of the amount of C used from structure and defense (Table 1).

The initial cost of the branch production (Eq. 17) is equivalent to the costs of the C and N used in producing the branch and any structural defenses, with branch structural defenses amortized over the number of years that the defenses are associated with leaves produced from the same meristem. For example, if the meristem on a short shoot produces leaves for 5 years, the initial costs of the branch defenses are spread out over that period. The C cost of the branch (Eq. 21) is equivalent to the amount of C allocated to the branch for defense, structure, and growth respiration. As in leaves, growth respiration (Eq. 22) is a constant fraction of the C content of the branch (both structure and defense). The N cost of the branch (Eq. 23) is equal to the N content of the branch multiplied by the relative value of C and N.

Herbivory and defense costs

The cost of herbivory (Eq. 24) is the cost of having leaves eaten. Here we parameterize herbivory rates both with the fraction of leaves and branches that are eaten (analogous to an herbivory rate) and the number of days into the growing season that the average leaf is eaten. This substitutes for a more complex relationship that describes the probability that a shoot would be browsed. The cost of leaf herbivory is equal to the sum of the lost C income due to herbivory, the depreciated value of the leaves eaten (except N that would have been resorbed), and the full cost of the N in the leaves that would have been resorbed. The lost C income represents the C that would have been fixed while leaves are being replaced (assuming that leaves will always be immediately replaced). Lost C income is equal to the fraction of the leaves that are eaten (fraction of the shoots that are browsed multiplied by the fraction of leaves

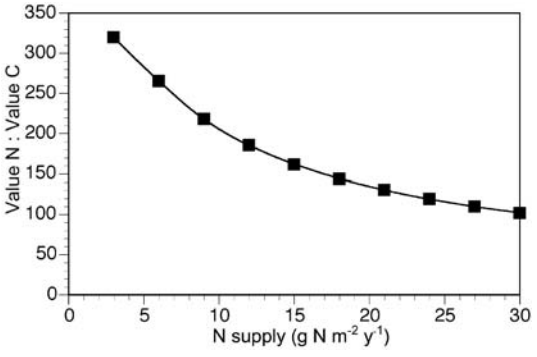

Fig. 1 Theoretical relationship between the relative value of N and C and ecosystem N supply. Values of N and C were derived from N mineralization and gross photosynthesis of a hypothetical N gradient. Gross photosynthesis was calculated in a general model of stand photosynthesis (PnET-Day) as a function of leaf area index and %N, derived from empirical relationships from temperate deciduous forest

that are eaten per unit shoot browsed) multiplied by the time it takes to replace a leaf and a leaf's expected net daily C gain.

If a leaf has a set lifespan, the value of the C and N in the leaf that cannot be remobilized declines with increasing leaf age as potential cumulative leaf productivity declines concomitantly. Therefore, leaves that are eaten are considered a loss, but the value of the leaves needs to be depreciated with time. For example, a leaf eaten the day before it was scheduled to be senesced would only have provided 1 more day's worth of photosynthesis before most of it was discarded anyway. No leaf structural C can be remobilized and all of its value is depreciated with time. Yet, a portion of the N can be remobilized. When a leaf is eaten by a herbivore, the value of the N that can not be resorbed is depreciated and the N that could have been remobilized is charged at full replacement value. Depreciation is a linear function of age, i.e., the value of non-salvageable resources decline linearly with age.

The rates of herbivory on leaves and branches are dependent on the type of defense and the amount of defense relative to the N content of the leaves and branches. We investigate the costs and benefits of three major defense strategies in addition to a control non-defended plant. For each defensive strategy, we examine the consequences of relatively high levels of investment. For tannins, additional C is allocated to leaves and stems in proportion to the amount of N in the leaf. Tannin concentrations were set to 10 g C in tannin per 1 g N in the leaf. Tannin levels ranged from 0.17 to 0.29 g C for a standard 1 g (dry) leaf, constituting 14–20% of leaf biomass. We also examine the economics of tannins as a defense except the maximum tannin content of leaves is set to 0.22 g C g^{-1} standard leaf (~17% final leaf biomass). A tannin:N ratio of 10 is assumed to confer full protection from herbivory and the fraction of leaves eaten is proportional to the square of the tannin:N ratio [10 −(C leaf defense/N leaf)^2 ×15]. This provides a reasonable range of herbivory rates from 0 to 81% of the leaf biomass eaten.

For N-based defense, we assume that a minimum content of defensive compound confers full defense. Shoots defended with N-containing compounds allocate an additional 0.02 g N and 0.40 g C per unit of shoot across all N-supply rates (Gleadow and Woodrow 2000). This is higher than levels found in leaves, but not uncommon for buds and reproductive parts. As it is part of our goal to understand why plants do not have extreme strategies, we have relaxed this apparent constraint on leaf concentrations of N-containing defenses. Later, we examine the consequences of having moderate amounts of N-containing defenses at low and high N supply. As the literature on the degree of turnover of the C skeletons of N-containing defenses is mixed, we assume the C in the N-based defenses turns over every 36 days, but the N is recycled.

Structural defenses allow a proportion of leaves to be browsed, but protect branches from being consumed. We use a generic structural defense that can represent both spines and the wire-plant strategy. A structurally defended plant has greater branch investment, leaves are produced on short shoots such that the same defense is reused for multiple years, yet leaves are browsed. Whether resources are invested into spines on branches or into the branch itself (along with longer distance between nodes) does not need to be explicitly incorporated into the calculations for the generic herbivory scenario that we utilize. As there is a lack of data on the costs and benefits of structural defenses, we assume branch C and N content increases 100%, which results in only 30% of the leaves being eaten, and the defenses are active for 3 years.

Sensitivity analyses

To understand the importance of including the value of N and having it change across an N supply gradient, we examine the net C gain of shoots defended by tannins and cyanogenic glycosides across an N supply gradient: (1) as described above, (2) with no cost of N, and (3) for a constant relative value of N:C (162, which is the relative value at 15 g N m^{-2} $year^{-1}$). As there are many parameters that could be altered, we perform two sensitivity analyses to investigate the relative importance of different parameters on net C gain. First, we examine the relationship between a suite of parameters and net C gain, by incrementally increasing (and decreasing) it until the parameter's value is double (and half) the original value. The sensitivity analysis is done at intermediate N supply (10 g N m^{-2} $year^{-1}$) with a set herbivory rate of 50% of the leaves eaten. The sensitivity analysis is performed for undefended plants, plants defended with tannins (with a maximum tannin content), N-based defenses, and structural defenses. We also examine the sensitivity of net C gain to different levels of defensive investment for the three defensive strategies.

Results

The relative costs of defenses without herbivory

In the absence of herbivory, structural defenses are generally the least expensive defenses to produce, requiring only 9.0% of the annual C gain of an undefended leaf at low N supply (3 g N m^{-2} $year^{-1}$) and 3.1% at high N supply (30 g N m^{-2} $year^{-1}$) (S, Fig. 2a). Short shoots are an important component in reducing the cost of the defense. Although spines increase the cost of branch production from 0.96 g C to 1.93 g C (an extra 22 days of photosynthesis) at low N supply and from 0.77 g C to 1.53 g C (an extra 12 days of photosynthesis) at high N supply, these costs are spread out over multiple years since the short shoots that are associated with the branch biomass reuse the same defense biomass multiple years. Reusing the same structural defense for 3 years (the case examined here) reduces the cost of the structural defense from 0.97 g C to 0.32 g C (saving the C equivalent of 15 days of photosynthesis) at low N supply and from 0.76 to 0.25 g C (savings of 8 days photosynthesis) at high N supply.

Tannins are the second least expensive defense strategy across the entire N-supply gradient (T, t, Fig. 2a). Producing a tannin C:leaf N ratio of 10:1 (10 mg C in tannin per 1 mg N in leaf) uses 7.1% (low N supply=3 g N m^{-2} $year^{-1}$) to 5.2% (high N supply=30 g N m^{-2} $year^{-1}$) of the net C gain of the equivalent amount of control leaf. Though the C content of the tannin in the leaf was equal to 38% and 64% of leaf structural C, this amount is still small compared to the 7.92 and 11.16 g C of net photosynthesis over the growing season for a leaf. Less expensive is the tannin strategy where there is a maximum tannin content, especially at high N supply, since the absolute amount of resources allocated to defense is constant at higher N supply but the net C gain for the leaf increases (only 4.0% of net C gain of the control).

The N-based defense is the most expensive defense at low N supply, costing 8.02 g C, which is 4.44 g C more than the standard shoot's net photosynthesis over the growing season (an extra 100 days of photosynthesis) (N, Fig. 2a). At the highest N supply, this defense strategy reduces net C gain of the shoot by 5.82 g C, which is 70% of the total net C gain. Without resorption of half of the defensive N, the N defense would have cost 3.9 times more than the net C gain of an undefended leaf at low N supply and reduced the C gain of the leaf by 100% at high

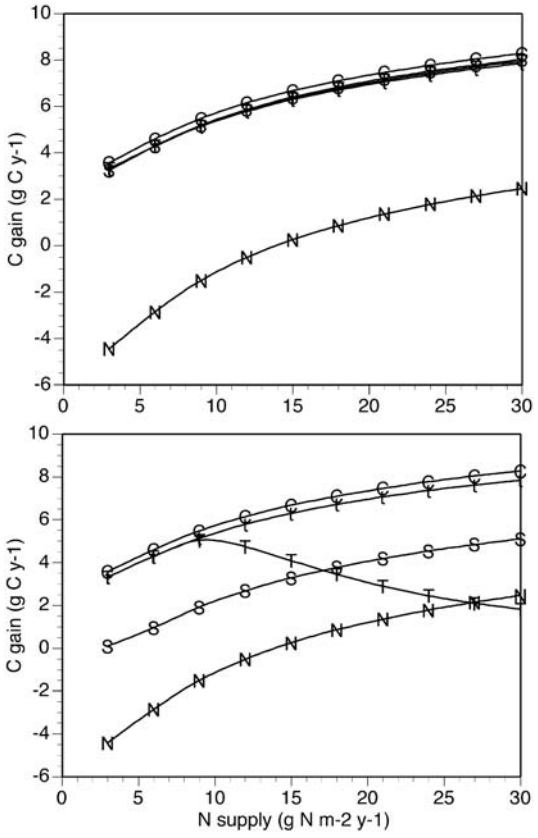

Fig. 2 Relationships between net C gain and N supply for shoots defended with tannins [both with (*T*) and without (*t*) a maximum tannin content], N-containing compounds (*N*), branch structural defenses (*S*), and undefended shoots (*C*) under scenarios without herbivory (*upper graph*) and with herbivory (*lower graph*). In the*lower graph*, undefended shoots experience no herbivory and are presented as a reference more than a representation of the patterns seen in natural conditions

N supply. The majority of the cost of the N-based defense is derived from the high cost of N, while the cost of the C was 13% and 21% of the total initial defense cost at low and high N supply, respectively. Turnover of the N defense 5 times during the year required an additional 4.0 g C (90 and 64 days of photosynthesis at low and high N supply, respectively).

Relative costs of defenses with herbivory

When herbivory scenarios for the different defenses are included, maintaining high tannin:N ratios across the N supply gradient provides the highest C gain of all defenses (T, Fig. 2b). The net benefit of tannins does not change with herbivory rates set to zero when maintaining a sufficient tannin:N ratio. Structural defenses require almost the entire net C gain of the leaf at low N supply (net C gain=0.10 g C) even with a low herbivory rate. At high N supply, structural defenses provide 62% of the C gain of an undefended shoot that has no leaf removal. At low N supply, C demand for the production of the structural defenses is too high to be met by the leaves, which have low photosynthetic capacity in addition to the costs of herbivory. These structurally defended shoots can only be justified at higher N supply where leaves have higher N concentrations and higher photosynthetic potential. Like structural defenses, N-based defensive compounds are not effective until an N supply of ~14 g N m^{-2} $year^{-1}$, though for different reasons. In the case of N-containing defensive compounds at low N supply, the C costs of acquiring the N that is used for defense are too large to be met by leaves with low N concentrations and lower photosynthetic potential.

If there is a maximum tannin content for a leaf and herbivory increases proportional to the ratio of N to tannin, then defense with tannins is still the most effective strategy at low N supply but less rewarding than the other defenses at higher N supplies (t, Fig. 2b). This strategy becomes less productive than structural defenses and N-containing defenses at intermediate–high N supply.

Sensitivity of leaf C gain to changes in parameters

We examined the influences of not including: (1) the relative value of C and N, and (2) the value of N in our analyses of net C gain of defended shoots for tannins and N-based defenses. Not including the relative value of C and N markedly affects the change in net C gain over the N supply gradient (Fig. 3), leading to a flatter relationship between N supply and net C gain for both defense types. Not including the cost of acquisition of N leads to greater net C gain for both tannins and N-based defenses.

The sensitivity analyses show that leaf C gain is most sensitive to factors that affect the relative value of C and N (the slope of the relationship between stand C gain and N supply) and the rate of C return for N in leaves (e.g., the slope and intercept of the photosynthesis-N relationship) (Fig. 4). The fraction of the leaves that are eaten strongly affects net C gain since the number of leaves eaten changes N loss and C return for leaves. Similarly, the age a leaf is eaten determines its lifetime C return.

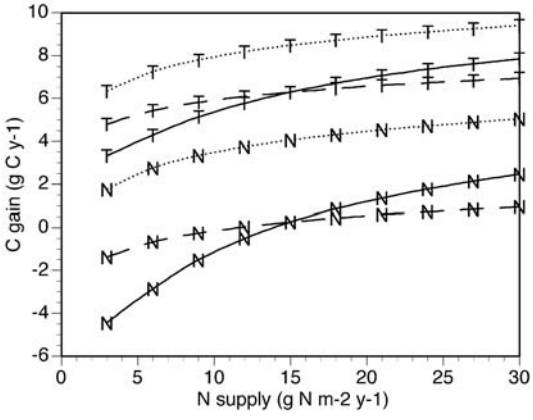

Fig. 3 Effect of not including any value for N (···) and a constant relative value of N and C (- - -) for two defense strategies (T and N) relative to a declining relative value of N:C with increasing N supply as in Fig. 2. For abbreviations, see Fig. 2

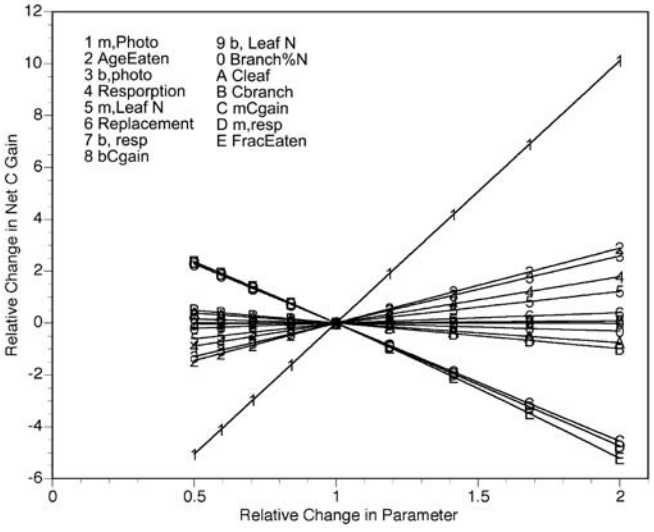

Fig. 4 Sensitivity analysis of net C gain of an undefended shoot that has 50% of its leaves eaten during the growing season at a common N supply (15 g N m^{-2} $year^{-1}$). Default values of parameters were varied from 0.5 to 2 times the original value and net C gain calculated for each case

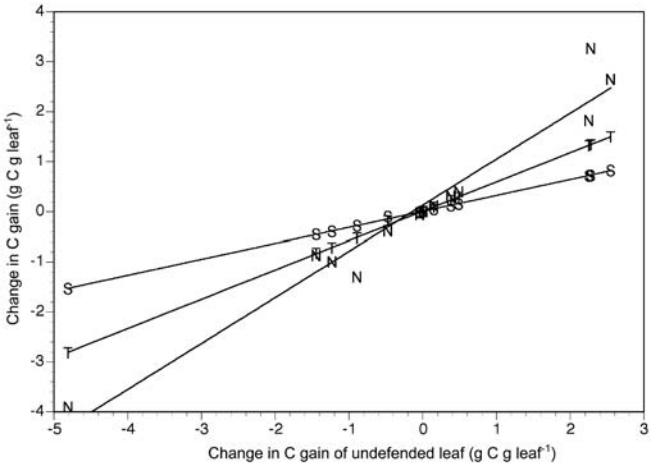

Fig. 5 Differences in sensitivity of net C gain to doubling of parameter values for three defense strategies (T, N, and S). For each parameter for which we examined the sensitivity of net C gain of an undefended leaf, we examined the sensitivity of net C gain of defended shoots to a doubling of the original parameter value. For abbreviations, see Fig. 2

Net C gain is relatively insensitive to the C content of leaves and branches. These factors involved relatively little amounts of C and no N, which would have a high exchange rate relative to C. Since daily photosynthesis is a small fraction of the total lifetime photosynthesis of leaves, altering leaf replacement by a factor of 2 (−7 or +14 days) results in little difference in lost C income. The intercepts of the photosynthesis-N, leaf N-N supply, and C gain-N supply relationships are all low values to begin with, and varying them by a factor of 2 has little effect on the net C gain of a shoot.

The sensitivity of net C gain of undefended shoots, and shoots defended with tannins, N-containing compounds, and structural defenses in the absence of herbivory to halving and doubling of parameters was virtually identical for all defense types (r >0.99, P <0.0001) (Fig. 5). Net C gain was more sensitive to changes in factors for shoots defended with N-containing compounds due to the high value of N.

Examining the sensitivity of net C gain of shoots with different defenses at 15 g N m^{-2} $year^{-1}$, shows relatively low sensitivity to parameters for C-based defenses, but high sensitivity for the N-containing defense. Plants defended with spines were relatively insensitive to changes in the longevity of structural defenses or the amount of C in the defense. Branch costs were 1.35 g C when short shoots reused spines for 1 year and 0.74 g C when they reused spines for 10 years. With structural defenses reused for 3 years, halving and doubling the C content of the structural defense required 0.11 g C less and 0.22 g C more per year, respectively. Plants defended with tannins were also insensitive to changes in the amount of C used in defense. Doubling defense investment for a plant at 15 g N m^{-2} $year^{-1}$ only adds an extra 0.25 g C, and halving it requires 0.13 g C less. Since N is generally very expensive in terms of C, plants defended with N-based compounds were very sensitive to halving and doubling defense N. Doubling the amount of N used in the leaves for defense decreased net C gain by 3.75 g C and halving it increased net C gain by 7.49 g C.

Discussion

Our analysis shows that, in general, the relative costs and benefits of different defense strategies match up well with their relative abundance along N-supply gradients and predictions based on the relative costs of C and N. Sites with low N supply are characterized by plants high in C-based defensive compounds and we found these compounds provide the greatest net returns at low N supply. High-N-supply sites are characterized by structural defenses or N-containing defensive compounds and these defensive strategies were generally not sustainable at low N supply, only leading to a positive resource income at higher N supplies.

Yet, contrary to the distributions of defenses among plants along N-supply gradients and predictions of resource availability theory, tannins appear to be an inexpensive defense at high N supply. Compared to the total amount of C that is fixed by a leaf over a growing season, the C required for tannins is relatively small. If high concentrations of tannins could be stored with little additional costs, according to our model the tannin strategy should be effective in deterring herbivory. A relatively small amount of C invested into tannins provides a high enough tannin:N ratio to limit protein assimilation by herbivores. In contrast, N-based defensive compounds cost many times more, even at high N supply where N has its lowest value relative to C. Structural defenses may not cost a lot of resources, especially when amortized over multiple years, but they still allow the

leaves to be eaten. The costs of herbivory associated with lost C income and lost N make losing leaves an expensive strategy. The observed high abundance of structural defenses in ecosystems with high N supply suggest that the protection afforded meristems and the consequent ability to reproduce leaves quickly leads to greater resource gain than we calculated here and/or that other defensive strategies are less effective than we assumed. If branches were browsed for other defensive options, the relative advantage of structural defenses would likely increase.

Only when there is a maximum tannin content to leaves and leaves are eaten do tannins become a less profitable defense than others at high N supply. Currently, there is no definitive research regarding the relationship between tannin concentrations and natural N-supply gradients or leaf protein concentrations, nor constraints on tannin concentrations in leaves at high N supply. Although a specific mechanism that may limit tannin accumulation in plant tissues is unknown, tannins are stored in specialized cells whose production may greatly increase the costs of the defense. In addition to increasing the cost of the defense over its synthesis costs, the presence of tannin stored in cells may disturb photosynthesis or respiration at high concentrations (Skogsmyr and Fagerstrom 1992).

Differences in leaf longevity with N supply may alter the relative benefits of C-based defenses. It has been hypothesized that at high resource availability, mobile defenses are favored since leaf longevity is often less (Coley et al. 1985). If, as shown by Reich et al. (1997), leaves at high N supply live for substantially shorter periods of time, tannins may become more expensive since their constituent resources can not be remobilized. We assumed that leaves have a set lifespan (180 days) in the absence of herbivory, regardless of N supply. Future empirical research needs to quantify the relationship between leaf longevity and defense strategies, while theoretical research should examine the consequences of differences in leaf longevity for the net benefits of different defense strategies.

Including the relative costs of C and N reveals why earlier research regarding N-containing defenses contradicted carbon-nutrient-balance-type hypotheses (Skogsmyr and Fagerstrom 1992). N-based defenses are expensive, mostly due to the high cost of N, but also due to the compound's high rate of turnover. If the relative value of N was constant across the N supply gradient, there would be a flatter relationship between N supply and net C gain. Data on the turnover rates of different N-containing defensive compounds are rare. The N in nicotine, an alkaloid, has been shown not to turn over as rapidly as previous analyses have suggested (Baldwin et al. 1994). Yet, even if the N in the compound has a low level of replacement, the C costs of maintaining the compound may be higher. Additional loss pathways such as volatilization of compounds could increase the cost of the defensive strategy, and once quantified should be included in future economic analyses of defenses.

For our analyses, we used a relatively high concentration of N-containing defensive compound based on the concentrations that can be found in buds and reproductive parts (Schroeder 1986). Our analyses show quite clearly that these high levels are not sustainable in leaves at low N supply due to the high cost of N. Yet, if a higher fraction of the N used in defense compounds is resorbed, the cost of the defense would decrease dramatically. N-containing defense compounds in leaves are often found at concentrations an order of magnitude less than those in buds and reproductive parts (Gleadow and Woodrow 2000). At these levels, (0.002 g N g leaf^{-1}), the defensive costs of N-containing defenses are only slightly more than tannins and spines, even at low N supply (data not shown).

While the relative value of C and N sets the constraints for the type of defensive strategies that can be used across an N-supply gradient and the level of investment into defenses, other factors must be incorporated into a complete theory of defensive allocation. As the costs of defensive strategies are clearly dependent on the level of defensive investment, more research is necessary to understand the optimal level of investment in defenses. Therefore, relationships between defense allocation and the functional response of herbivores need to be quantified carefully. The amount of leaf herbivory has a strong effect on the net C gain of shoots. Herbivory of leaves not only reduces C return per unit N invested, but also decreases the amount of N that can be recovered from the leaves.

Although we assume that maintaining a sufficiently high level of investment into a defensive strategy limits herbivory at least partially if not entirely, every defensive strategy can be circumvented to a degree (e.g., Schappert and Shore 1999). For tannins, insects such as gypsy moths (*Lymantria dispar*) have a high midgut pH plus surfactants, which together limit the ability of tannins to bind to protein (Schultz and Lechowicz 1986, De Veau and Schultz 1992). Many insects are able to sequester N-containing glycosides without damaging effects (Hartmann et al. 1999). Large thorns and spines are generally ineffective against insects and the wire-plant defense that protected New Zealand woody plants from ratite browsing are ineffective against mammals (W. Bond et al., unpublished data). As such, evolution of defense systems needs to incorporate the evolutionary probability of being eaten (Adler and Karban 1994). Plants most likely hedge investments by including multiple defensive strategies. For example, plants with thorns can have tannins (Brooks and Owen-Smith 1994). *Prunus serotina* (black cherry) leaves have appreciable quantities of both tannins and cyanogenic glycosides (Schroeder 1986).

Sensitivity to parameters

It is clear from the sensitivity analysis that understanding the patterns of defenses and plant growth along N-supply gradients requires constraining the relative value of C and N. As net C gain accounting for N costs was most sensitive to the relative value of C and N, future research

that focuses on the relative costs and benefits of defenses needs to emphasize and quantify the relative value of constituent resources. Although ecosystem supply of C relative to N is high at low N supply, it is important to note that C is expensive for plants to acquire at low N supply. It requires very expensive N to photosynthesize and plants are likely to be more conservative with both C and N at low N supply. Likewise, at high N supply, photosynthetic rates are higher than at low N supply and plants not only acquire more N, but also more C. Although we analyzed the sensitivity of net C gain to changes in parameters by a factor of 2, total sensitivity will need to incorporate the full range in potential parameter values. As such, the ranking of the importance of factors in determining net C gain may be different when the full range of values for parameters is incorporated.

Our assumptions of the relative value of C and N will have to be adjusted when considering N_2-fixing plants, which are known to use N-based defensive chemicals in habitats with low N mineralization. Our calculations assume the relative values of N and C are dependent on generalized stand-level photosynthesis relationships for non N_2-fixing plants that show asymptotic increases in C fixation with increasing N supply. N_2-fixing plants like legumes are likely to have more photosynthesis at a lower external N supply, due to higher leaf N supplied by fixation. This results in cheaper N at low N supply and more abundant N-based defenses in fixers at low N supply than non-fixers (Coley and Barone 1996). Similarly, since N is less (or not at all) limiting for P-limited plants, N-containing defensive compounds are more likely to be present in higher concentrations than if N was limiting. For example, *Eucalyptus cladocalyx* , which is likely to be P-limited in its native habitat and as a consequence can have low leaf-N concentrations, can allocate up to 20% of leaf N to cyanogenic glycosides (Gleadow and Woodrow 2000).

Conclusions

By extending economic models of shoot resource balance to include the relative value of C and N, depreciation, and amortization, we were able to show how the costs and benefits of defense types changed across an N-supply gradient. The relative net benefits of different defense strategies are highly dependent on ecosystem N supply and the relative value of C and N.

At low N supply, N is too expensive in terms of C to be used for defense. Similarly, structural defenses require too much C for low-N leaves to photosynthesize and support the rest of the plant, especially since leaves are not completely protected. C-based defenses are inexpensive at low N supply, requiring only a small amount of C to protect a leaf. C-based compounds appear inexpensive at high N supply too, and either have additional costs that make them less favored or for some reason lower tannin: protein ratios are required at higher protein concentrations. At high N supply, N becomes cheaper and more favorable economically to use for defense. Since total C uptake also increases at high N supply, structural defenses become more favored, both because C is abundant and the N lost from leaves costs less.

Future research will need to address the economics of tannins at high N supply and N-containing compounds at low N supply. More generally, our certainty in average parameters is low, no less how they may change across resource gradients. More data needs to be collected in this area and models will need to accommodate different environmental constraints on plant growth, such as shading that occurs with canopy closure, as well as the functional responses of herbivores.

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Appendix 1

Formulae used to calculate net C gain of shoots

$$\mathrm{NetCGain} = \mathrm{LeafCGain} - \mathrm{CostInitialShoot} + \mathrm{Salvage} - \mathrm{CostHerbivory} \quad (1)$$

$$\mathrm{LeafCGain} = \mathrm{Cgainday} \times \mathrm{LongevityLeaf} \quad (2)$$

$$\mathrm{Cgainday} = \mathrm{Photo} - \mathrm{Resp} \quad (3)$$

$$\mathrm{Photo} = k_{\mathrm{photo}} \times m_{\mathrm{photo}} \times \%\mathrm{NLeaf} + b_{\mathrm{photo}} \quad (4)$$

$$\%\mathrm{NLeaf} = \ln(\mathrm{Nsup}) \times m_{\mathrm{Nleaf}} + b_{\mathrm{Nleaf}} \quad (5)$$

$$\mathrm{Resp} = (\mathrm{NBranch} + \mathrm{NLeaf}) \times \mathrm{RespCoeff} \quad (6)$$

$$\mathrm{NBranch} = \%\mathrm{NBranch} \times \mathrm{CBranchStructure} \quad (7)$$

$$\mathrm{CBranch} = \mathrm{CBranchDefense} + \mathrm{CBranchStructure} \quad (8)$$

$$\mathrm{NLeaf} = \%\mathrm{NLeaf} \times \mathrm{CLeafStructure} + \mathrm{NLeafDefense} \quad (9)$$

$$\mathrm{LongevityLeaf} = \mathrm{FracShootEaten} \times \mathrm{FracLeafEaten} \times \mathrm{LeafAgeEaten} + [1 - (\mathrm{FracShootEaten} \times \mathrm{FracLeafEaten})] \times \mathrm{LongevityLeafMax} \quad (10)$$

$$\mathrm{Salvage} = (1 - \mathrm{FracShootEaten})(\mathrm{FracLeafEaten}) \times \mathrm{CostNLeaf} \times \mathrm{Resorption} \quad (11)$$

$$\mathrm{CostNLeaf} = \mathrm{NLeaf} \times \mathrm{ValueC/N} \quad (12)$$

$$\mathrm{ValueC/N} = \mathrm{CgainStand/Nsupply} \quad (13)$$

$$\text{CgainStand} = m, \text{Cgain} \times \ln(\text{Nsupply}) + b, \text{Cgain} \quad (14)$$

$$\text{CostShootInitial} = \text{CostLeafInitial} + \text{CostBranchInitial} \quad (15)$$

$$\text{CostLeafInitial} = \text{CostCLeaf} + \text{CostNLeaf} \quad (16)$$

$$\text{CostCLeaf} = \text{CLeaf} \quad (17)$$

$$\text{CLeaf} = \text{CLeafDefense} + \text{CLeafStructure} + \text{CLeafGrowthResp} \quad (18)$$

$$\text{CLeafGrowthResp} = \text{RespCoeff} \times (\text{CLeafStructure} + \text{CLeafDefense}) \quad (19)$$

$$\text{CostBranchInitial} = \text{CostCBranch} + \text{CostNBranch} \quad (20)$$

$$\text{CostCBranch} = \frac{\text{CBranchDefense}}{\text{BranchDefLong}} + \text{CBranchStructure} + \text{CBranchGrowthResp} \quad (21)$$

$$\text{CBranchGrowthResp} = \text{RespCoeff} \times (\text{CBranchStructure} + \text{CBranchDefense}) \quad (22)$$

$$\text{CostNBranch} = \text{NBranch} \times \text{ValueC/N} \quad (23)$$

$$\begin{aligned}\text{CostHerbivoryLeaf} = {} & \text{FracLeafEaten} \times \{(\text{ReplacementLeaf} \times \text{Cgainday}) \\ & + \left[\left(1 - \frac{\text{LeafAgeEaten}}{\text{LongevityLeafMax}}\right) \times (\text{CostCLeaf} + \text{Resorption} \times \text{CostNLeaf})\right] \\ & + [(1 - \text{Resorption}) \times \text{CostNLeaf}]\}\end{aligned} \quad (24)$$

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