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Herbivory and plant tolerance: experimental tests of alternative hypotheses involving non-substitutable resources

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A mechanistic understanding of the highly variable effects of herbivores on plant production in different ecosystems remains a major challenge. To explain these patterns, the compensatory continuum hypothesis (CCH) predicts plants to compensate for defoliation when resources are abundant, whereas the growth rate hypothesis (GRH) makes the opposite claim of high herbivory tolerance under resource-poor conditions. The limiting resource model (LRM) tries to reconcile this dichotomy by incorporating the indirect effects of herbivores on plant resources and predicts that the potential for plant compensation is dependent upon whether, and how, herbivory influences limiting resources. Although extensively evaluated in laboratory monocultures, it remains uncertain whether these models can also explain the response of heterogeneous and multi-species natural plant communities to defoliation. Here we investigate community-wide plant response to defoliation and report data from a field experiment in the arid and primarily water-limited Trans-Himalayan grazing ecosystem in northern India involving clipping, irrigation and nutrient-feedback with herbivore dung. Without nutrient-feedback, plants compensated for defoliation in absence of irrigation but failed to compensate under irrigation. Whereas, in the presence of nutrient-feedback plants compensated for defoliation when irrigated. This divergent pattern is not consistent with the CCH and GRH, and is only partially explained by the LRM. Instead, these pluralistic results are consistent with the hypothesis that herbivory may alter the relative strengths of water and nutrient limitation since irrigation increased root:shoot ratio in absence of fertilization in unclipped plots, but not in the corresponding clipped plots. So, herbivory appears to increase relative strength of nutrient-limitation for plants that otherwise seem to be primarily water-limited. Extending the LRM framework to include herbivore-mediated transitions between water and nutrient-limitation may clarify the underlying mechanisms that modulate herbivory-tolerance under different environmental conditions.

Plant–herbivore interactions involving defoliation and tissue loss have conventionally been considered antagonistic – herbivores may have negative impacts on plant growth and fitness. Much research has focused on traits that reduce or deter herbivory (i.e. resistance), such as secondary structures and chemicals (Crawley 1983). In comparison, less appears to be known about herbivory-tolerance due to mechanisms that compensate tissue-loss following defoliation, and this represents a major area of research since it has significant applied importance for land management (Levin 1993, Juenger and Lennartsson 2000). Empirical studies indicate that herbivores, on average, consume about one-fifth of plant production in terrestrial ecosystems (Cyr and Pace 1993). In doing so, they can have positive, neutral, or negative effects on plant production, depending on the study system (reviewed by Milchunas and Lauenroth 1993). Such variable effects may arise due to multiple overlapping mechanisms that are thought to be involved in determining herbivory-tolerance in plants: (1) photosynthetic rate, (2) growth rate, (3) release from apical dominance, and (4) the translocation of stored photosynthates after defoliation (Strauss and Agrawal 1999).

For a long time, it has generally been assumed that plant tolerance to herbivory is favored by resource-rich and benign environmental conditions (Mueggler 1967). Maschinski and Whitham (1989) formalized this viewpoint as the 'compensatory continuum hypothesis' (CCH). The primary assertion of the CCH is that a greater potential for compensation (or overcompensation) of tissue loss exists under resource-rich conditions, compared to more stressful and resource-poor conditions. However, counter-intuitively, several studies have documented an inverse association between resource availability and herbivory-tolerance (reviewed by Hawkes and Sullivan 2001). Hilbert et al. (1981) formalized this aspect as the 'growth rate hypothesis' (GRH) to predict that a greater potential for herbivory-tolerance exists under relatively stressful and resource-poor conditions, compared to resource-rich conditions. Central to the GRH is the argument that plants maintain a slow growth rate under stressful conditions relative to the potential maximum rate under the same conditions. After defoliation they can accelerate their growth rate to full potential and may compensate for tissue loss, relative to corresponding controls. However, under more favorable conditions plants are already near their

maximum potential growth rate, and cannot increase it any further. Therefore, they are unable to compensate for tissue loss relative to corresponding controls (Hilbert et al. 1981). Wise and Abrahamson (2005) attempted to resolve this apparent dichotomy of viewpoints (i.e. CCH vs GRH) by proposing the 'limiting resource model' (LRM). The major advance under this LRM framework is that it specifically considers whether herbivory may influence the availability of a key limiting resource, or that of alternative resources which are presumed to be non-limiting (i.e. Liebig's law, De Baar 1994). Hence, a wide array of outcomes ranging from under- to overcompensation is predicted by the LRM, contingent upon which resource is affected by herbivores (Wise and Abrahamson 2005). In this manner, the LRM is thought to be a pluralistic and flexible framework that encompasses multiple contingent models (Banta et al. 2010).

The formal mathematical theory linking herbivore effects to resource availability adheres more closely to the LRM framework, than either the GRH or CCH (Loreau 1995, de Mazancourt et al. 1998, de Mazancourt and Loreau 2000). The predictions of these mathematical models are related to positive feedback pathways arising from the indirect effects of herbivores on nutrient cycling. Herbivores can influence nutrient cycling by converting plant tissue to dung and urine, and altering the quality of plant tissue via physiological responses and/or compositional turnover (Ritchie et al. 1998, Chapman et al. 2003). Among slow-growing and well-defended plants that produce slow-decomposing litter, nutrient cycling is depressed by herbivores, resulting in a low potential for herbivory-tolerance. Alternatively, among fast-growing plant species that produce readily decomposable litter, nutrient cycling is accelerated by herbivores (Ritchie et al. 1998, Chapman et al. 2003), resulting in a high potential for herbivory-tolerance.

A powerful method to test these proposed mechanisms that may underlie herbivory-tolerance involves measuring plant performance under combinatorial arrangements of altered resource supply and differential tissue damage, particularly when defoliation occurs early in the growth season (Strauss and Agrawal 1999). Many single-resource manipulative experiments have been conducted under laboratory conditions on plant monocultures (Hamilton et al. 1988); and have found evidence supporting either the GRH or the CCH, depending on the experimental conditions (reviewed by Hawkes and Sullivan 2001, Wise and Abrahamson 2007). Investigations of the LRM require multiple-resource manipulations, and the available evidence is primarily from reviews and meta-analyses of existing literature (Wise and Abrahamson 2007, 2008) and seldom from direct experimental tests (Banta et al. 2010). As very few studies have manipulated more than one resource (Strauss and Agrawal 1999), it has not been possible to fully distinguish the effects of limiting and alternative resources (i.e. Liebig's law, De Baar 1994). Further, there is a distinct dearth of multi-resource manipulative experiments under real-world conditions where plants do not grow as monocultures, but rather in multi-species communities. So, it remains uncertain whether these alternative theoretical frameworks can be seamlessly extended to include community-wide patterns of herbivory-tolerance, and thereby provide guidelines for developing and improving land management strategies. The average community-wide response of natural communities to defoliation, wherein edpahic conditions and plant species composition are inherently heterogeneous, may be highly relevant to real-world land-management concerns (Levin 1993, Juenger and Lennartsson 2000). However, unlike laboratory monocultures, it remains uncertain whether the average community-wide response to herbivory under natural field conditions can also be explained by the three alternative models.

We report data from a multi-resource manipulative experiment that evaluates community-wide herbivory-tolerance in plants growing under natural field-conditions. Here, plant response to defoliation can be influenced by – (1) plasticity in plant growth in relation to resource availability, and/or (2) species replacements – and theoretical models of plant–herbivore interactions have considered these linkages (de Mazancourt and Loreau 2000). Models of multi-species plant communities have shown that plant response to herbivory continues to be determined by resource conditions, even when species replacements occur (de Mazancourt and Loreau 2000), although different communities may have different levels of minimum resource requirements (Tilman 1982). When species replacements occur, then the responses of the new species are still subject to resource conditions in the same manner as the species which were displaced (Tilman 1982, de Mazancourt and Loreau 2000). Therefore, a multi-resource manipulative experiment under field conditions can provide useful insights into community-wide herbivory-tolerance based on alternative predictions concerning resource conditions (i.e. GRH, CCH, LRM).

Three non-substitutable resources are generally thought to be important in determining plant responses (Tilman 1982) – light, water, and soil nutrients (chiefly nitrogen, N, in most terrestrial environments). In relatively dry environments, competition for light is unlikely to be intense since production is often not high enough to cause shading (e.g. when plant biomass is below 300 g m^{-2}, Huisman and Olff 1998). Rather, plants are more likely to be limited by water or soil-N (Strauss and Agrawal 1999). Empirical patterns indicate that many terrestrial ecosystems are likely limited either by soil-N (Vitousek and Howarth 1991), or by water (Huxman et al. 2004), or both (Hooper and Johnson 1999). We implemented a manipulative experiment involving clipping (simulated herbivory), irrigation (simulated water input) and fertilization (simulated nutrient feedback via dung) in the high-altitude arid Trans-Himalayan shrub-steppe ecosystem where productivity is not high enough to induce potentially light-limited conditions for plants. Reviews have found that in this high altitude eco-region, aboveground plant biomass ranges between 40 and 194 g m^{-2} and belowground biomass ranges between 210 and 1254 g m^{-2} (Yang et al. 2010). Since the majority of total plant production occurs belowground, this may indicate strong limitation due to soil resources in this eco-region – water or soil-N (Hutchings and John 2004). Studies have also shown that the primary limiting resource is likely to be water as plant production in this eco-region is most sensitive to changes in precipitation (Christensen et al. 2004).

Under a full-factorial arrangement of clipping, irrigation, and fertilization, the different pair-wise multiple-comparisons of treatment means can help investigate the predictions of the CCH, GRH and LRM. The CCH would predict

equal- or overcompensation under irrigation or fertilization, compared to non-irrigated or unfertilized conditions. So, clipped production is expected to be equal to, or greater than, the corresponding unclipped production if plots are fertilized or irrigated. Alternatively, the GRH would predict under-compensation under irrigation and fertilization. So, clipped production is expected to be less than the corresponding unclipped production if plots are fertilized or irrigated. Predictions of LRM are not as straightforward due the inherent contingencies, as the outcome would depend on whether water or soil-N limits plant production. If water is the limiting resource, then LRM predicts irrigation to increase production in the clipped plots relative to unclipped plots, regardless of their fertilization status. If the alternative situation were true – soil-N is the limiting resource – the LRM would predict fertilization to increase production in the clipped plots relative to the unclipped plots, regardless of their irrigation status. Thus, the different predictions of the three alternative frameworks can be evaluated based on multiple-comparisons of the different treatment means in a full-factorial experimental design.

Material and methods

Study site

The Spiti region in northern India (12 000 km^2) is a part of the larger Trans-Himalayan landscape that covers 1.6 million km^2 in India, China and Nepal (an area larger than Alaska), and extends into Ladakh (northwards) and the Tibetan plateau (eastwards). The study was carried out in rangelands adjoining the village Kibber (32°N, 78°E), where climate is cold and arid with temperatures dropping below –30°C between November and March, allowing only a short growth season for plants (May–August). Vegetation is characterized by perennials including grasses, sedges, forbs and shrubs, while the tree layer is absent (Bagchi et al. 2006, Bagchi and Ritchie 2010). These rangelands are grazed by large herbivores including native species (yaks, *Bos grunniens*; ibex, *Capra sibirica*; and bharal, *Pseudois nayaur*), and various introduced grazers (cattle, donkeys, goat, sheep and horse). Conservation challenges facing native biodiversity, arising from such multiple-use, are discussed elsewhere (Bagchi et al. 2004, 2006, Bagchi and Ritchie 2010).

Experimental design

In May 2006, a field experiment was initiated to document plant responses to simulated herbivory through clipping over different conditions of resource-availability arising from application of herbivore-dung and through irrigation, inside permanently fenced 10 × 10 m herbivore exclosures (n = 9). Edaphic conditions did not show high levels of variation among these nine exclosure-sites as soil carbon content (C) was 1.28 ± 0.07%, soil-N content was 0.13 ± 0.01%, with soil C:N ratios of 9.85 ± 0.42. Soils were alkaline (pH of 7.73 ± 0.09), with sandy-loam texture (47.81 ± 1.52% sand), with bulk density of 1.01 ± 0.04 g cm^{-3}, and elevation was 4325 ± 31 m a.s.l. (Bagchi and Ritchie 2010). At each exclosure-site, we established two series of four plots, each 0.5 × 0.5 m, and adjacent plots were separated by a buffer of 0.5 m. We used a randomized design for each series, and fertilized two plots with dung of yak–cattle, and maintained two plots as respective controls. One plot of this pair was randomly assigned to the irrigation treatment. We assigned one of the two series to a clipping treatment which simulated herbivory, and maintained the other set as corresponding controls. This yielded a full factorial design with three treatments – clipping (two levels, clipped and unclipped) and irrigation (two levels, irrigated and non-irrigated), under two different regimes of nutrient-feedback from dung-application (two levels, fertilized and unfertilized). Each paired-series was replicated in the n = 9 exclosure-sites, each of which was characterized by sedge-meadow vegetation (dominated by *Carex melanantha*), with a total of n = 72 replicates.

In June 2006, we clipped one series of plots to 2.5 cm above ground level, and this clipping treatment was representative of the prevailing grazing intensity in this region (ca 40–50%, Bagchi and Ritchie 2010). We simulated nutrient-feedback using dung of yak–cattle since they are the dominant herbivores in this study area in terms of animal biomass. Fresh dung was collected from the rangelands and oven-dried for weighing (overnight at 40°C). These samples were gently ground to small pieces and mixed well. From this mixture, 50 g of dung was moistened with 50 ml of water and applied uniformly over each plot. Average C-content of this dung was 40.92 ± 0.84 % (Bagchi and Ritchie 2010) and N-content was 0.9 ± 0.06%. So, dung-application led to an average input of 1.8 g N m^{-2}. While this amounts to only moderate levels of N-amendment compared to some previous studies (Wilson and Tilman 1991), it is likely comparable to the strength of prevailing herbivore-mediated nutrient feedback in these rangelands since it attempts to simulate the natural dung-deposition levels (Bagchi and Ritchie 2010). We checked all plots carefully for any pre-existing dung, which we removed before experimental dung-application. For the irrigation treatment, we uniformly sprinkled 500 ml of locally available stream water over the target plots at weekly intervals for 12 weeks between June and August 2006. This amounts to simulated additional rainfall of about 24 mm, corresponding with ca 10% of natural precipitation (ca 200–300 mm annually).

Primary source of N-feedback in the unfertilized plots was plant litter that decomposes much slower than the dung which was available to the fertilized plots. Studies have shown that plant growth response is influenced by such implicit time-lags introduced by this difference in decomposition rates (Olofsson et al. 2007). So, an unbiased interpretation of patterns can be obtained only if the experimental duration accounts for this difference, allowing enough time for nutrients to be recycled by the slower pathway as well (Olofsson et al. 2007). Based on a pilot study to assess rates of litter and dung decomposition (Bagchi unpubl.) we ran the experiment for two growth seasons, to allow sufficient time for the slower litter decomposition pathway. Hence, we repeated irrigation, clipping and dung-application, as described above, in 2007. We estimated aboveground net primary production (ANPP) in August 2007 when all live aboveground biomass was clipped to ground level and oven-dried to obtain dry weight.

The available theoretical frameworks (CCH, GRH and LRM), and the mathematical models of herbivory-tolerance (de Mazancourt et al. 1998) do not explicitly incorporate that any aboveground response to herbivory may not be independent of the belowground compartment (Canham et al. 1996, Burke et al. 1998, Cahill Jr 1999). At the end of the study (August 2007), we estimated belowground biomass by sampling a 2.5-cm diameter, 20-cm deep soil-core taken from the centre of each plot, since more than 95% of roots are concentrated within 15-cm soil depth in these sedge meadows (Xu et al. 2004). Roots (including rhizomes and other associated structures) were washed in running water using a 1-mm sieve and oven-dried to obtain dry weights. Estimates of standing belowground biomass are not an unbiased reflection of net belowground production since they do not account for potential short-term root-turnover. However, estimates of belowground biomass may suitably represent the differences in production among the experimental treatments. We evaluated root:shoot ratios for the different treatment combinations to assess how the above- and belowground responses were inter-related.

Data analysis

Differences between treatments were determined through mixed-model analysis of variance (ANOVA) of main-effects and interactions in SAS 9.1 (SAS Inst.) with a full-factorial arrangement of three treatments – clipping, fertilization and irrigation – each with two levels. Data on root:shoot ratios required ln-transformation before analysis. Data are reported as mean ± 1 SE of dry weight. Multiple-comparisons between treatment means were conducted to detect compensatory growth in the treatments relative to corresponding controls using Tukey–Kramer adjustment (α = 0.05). While the alternative theories can be readily tested using controlled experiments with laboratory monocultures (Banta et al. 2010), sources of uncontrolled variation in natural plant communities may introduce potential confounding influences. For example, unlike laboratory monocultures, in natural multi-species plant communities it is difficult to a proiri control for local heterogeneity in edaphic conditions, or plant species composition, or plant genotypes. However, it is possible to evaluate the potential influence of such uncontrolled sources of variation a posteriori, and we verified our results using two approaches. First, we included exclosure-sites as a random factor in the ANOVA (i.e. blocking). Second, although our plots were all characterized by sedge-meadow vegetation with a small species pool, we assessed the potential influence of species compositional differences in our results. For this, we sorted the clipped biomass into four dominant functional groups – grasses (*Stipa orientalis*, *Elymus longae-aristatus*, *Leymus secalinus*), sedges (*Carex melanantha*), sub-shrubs (*Astragalus grahamiana*) and forbs (*Cousinia thomsonii*, *Lindelophia anchusoides*, *Nepeta discolor*, *Polygonum* sp.) – at the beginning (clipped plots, 2006) and the end of the study (all plots, 2007). Using multidimensional scaling (MDS) we ascertained whether compositional similarity (estimated as Bray–Curtis similarity index) varied systematically among the different treatments. If composition varied systematically among the treatment-combinations, then this would indicate that the results of multiple-comparisons of means in the ANOVAs were likely influenced by pre-existing differences in plant community composition.

Results

Aboveground production

ANOVA results showed a significant three-way interaction between clipping, irrigation and dung-application (Table 1). Multiple-comparison tests showed that in absence of nutrient-feedback, clipping promoted aboveground production in un-irrigated plots (overcompensation by +25%, Fig. 1a), but decreased it in irrigated plots (under-compensation by –30%, Fig. 1a). Alternatively, when nutrient-feedback due to dung-application was available, clipping yielded equal-compensation in non-irrigated plots, whereas, it led to over-compensation in the irrigated plots (by +20%, Fig. 1b). In the absence of clipping, irrigation led to increased production in the unclipped plots regardless of nutrient-feedback status (Fig. 1a–b). But in the clipped plots, irrigation led to increased production only when applied alongside fertilization (Fig. 1b). Whereas, in the clipped plots irrigation led to lower production in unfertilized plots (Fig. 1a).

Belowground biomass

ANOVA results indicated that only clipping and irrigation had significant main-effects, and there were no significant interactions (Table 2). Given the lack of significant interactions (Table 2), we re-analyzed these data using a reduced model with only the main effects. This reduced model also showed that clipping ($F_{1,60}$ = 6.07, p = 0.01) and irrigation ($F_{1,60}$ = 5.66, p = 0.02) had significant effects on belowground biomass. In absence of clipping, irrigation led to increased belowground biomass in the unfertilized plots (Fig. 2a). Also in absence of clipping, irrigation had no effects on belowground biomass in the fertilized plots (Fig. 2b). Among the clipped plots, irrigation led to increase in belowground biomass in the unfertilized plots (Fig. 2a), but it had no effect in the fertilized plots (Fig. 2b).

Table 1. Results of mixed-model ANOVA to test interactive effects of clipping, fertilization by dung-application, and irrigation on aboveground plant production in Spiti region of northern India. Site was included as a random factor. Given a significant three-way interaction, the results were further investigated with the help of multiple-comparisons of means.

Effect	DF	SS	MS	F	p
Site	8	652.87	81.61	1.86	0.08
Clipping	1	147.80	147.80	3.37	0.07
Fertilization	1	583.68	583.68	13.32	<0.001
Irrigation	1	740.61	740.61	16.91	<0.001
Clipping × Fertilization	1	375.20	375.20	8.57	0.005
Clipping × Irrigation	1	332.30	332.30	7.59	0.008
Fertilization × Irrigation	1	561.57	561.57	12.82	0.001
Three-way interaction	1	485.68	485.68	11.09	0.001
Error	56	2453.06	43.80		
Total	71	6332.78			

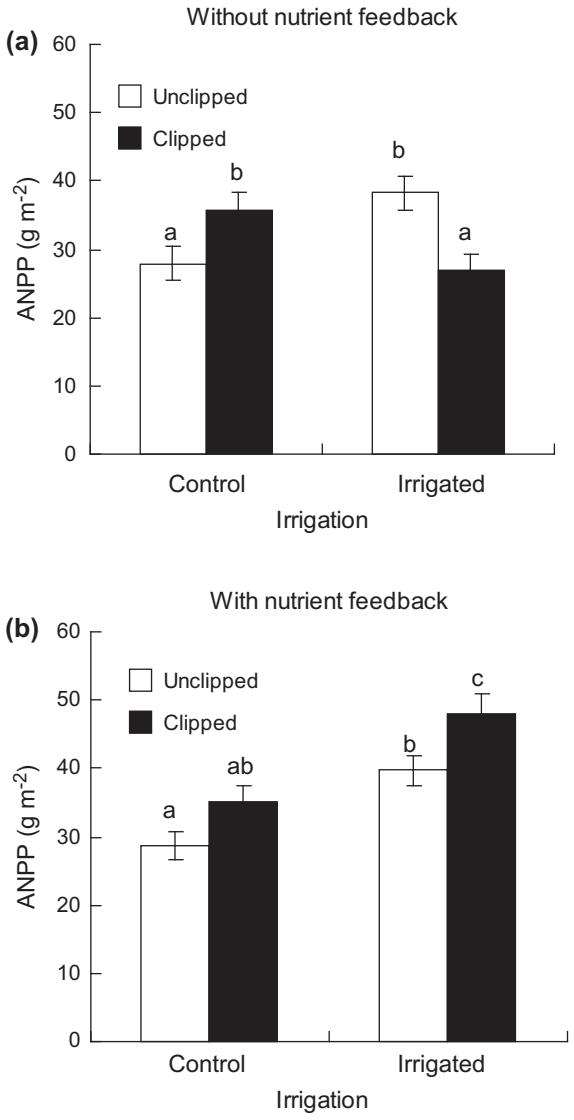

Figure 1. Mean (±1 SE) aboveground net primary production (g m^{-2}) of clipped and unclipped plots under two levels of water-availability (irrigated and non-irrigated). Results under the presence of nutrient-feedback pathways arising from dung-application (a) and in the absence of nutrient-feedbacks (b) are shown separately. Different letters show differences among means in a multiple-comparison test.

Root:shoot ratio

ANOVA results indicated that clipping and fertilization had significant main effects, while fertilization and irrigation had interactive effects on root:shoot ratio (Table 3). Clipping reduced root:shoot ratio by 23.8% and fertilization reduced it by 26.6% (Fig. 3a–b). Multiple comparisons of means for the interaction between fertilization and irrigation showed that irrigation in the absence of fertilization led to increased root:shoot ratio in the clipped plots (Fig. 3a), but not in the unclipped plots (Fig. 3b).

Composition effects

Results from MDS analyses show that while there were compositional differences among the plots, this variation was not systematically distributed among the different treatments, both in the beginning (Fig. 4a) as well as at the end of the study (Fig. 4b).

Table 2. Results of mixed-model ANOVA to test interactive effects of clipping, fertilization by dung-application, and irrigation on belowground biomass in Spiti region of northern India. Site was included as a random factor. Given a lack of significant interaction effects, the data were re-analyzed with a reduced model including only the main effects (see text for details).

Effect	DF	SS	MS	F	p
Site	8	35.73	4.46	3.94	0.001
Clipping	1	6.83	6.83	6.03	0.02
Fertilization	1	2.64	2.64	2.33	0.13
Irrigation	1	6.37	6.37	5.62	0.02
Clipping × Fertilization	1	0.08	0.08	0.07	0.79
Clipping × Irrigation	1	0.60	0.60	0.53	0.47
Fertilization × Irrigation	1	3.43	3.43	3.03	0.09
Three-way interaction	1	0.01	0.01	0.01	0.94
Error	56	63.45	1.13		
Total	71	119.13			

Discussion

Our primary objective was to evaluate whether the three alternative models could explain the response of natural multi-species plant communities to defoliation. Here, unlike laboratory monocultures, local heterogeneity in edaphic conditions and in community composition may potentially introduce uncontrolled sources of variation in our data. Using exclosure-sites as a random blocking factor in the ANOVAs (Table 1–3), may allow the multiple-comparison of treatment means (Fig. 1–3) to be viewed independent of natural heterogeneity in local growth conditions. Based on the results of vegetation composition it appears that while there was inherent heterogeneity in plant species composition, there were no systematic differences in species composition between plots assigned to the different treatments at the beginning of the study (Fig. 4a). Likewise, there was little evidence for systematic differences in species composition between the treatment combinations at the end of the study (Fig. 4b). So, it appears unlikely that pre-existing differences in species composition, and any subsequent compositional turnover during the study, may have introduced directional biases in the ANOVA results. This suggests that the multiple-comparisons of different treatments in the ANOVA (Fig. 1–3), that target specific predictions from the alternative models, are unlikely to be artifacts of pre-existing compositional differences or directional community shifts during the study. Instead, they may suitably represent the average community-wide response to the different treatments (de Mazancourt and Loreau 2000). And such average community-wide responses are perhaps relevant to real-world management scenarios (Levin 1993, Juenger and Lennartsson 2000).

Since there was a significant three-way interaction between the treatments for aboveground production (Table 1), differences in multiple-comparisons of treatment means (Fig. 1) indicate how the response to clipping may depend on both water (irrigation) and soil nutrients (dung). In absence of clipping, irrigation increased aboveground production in

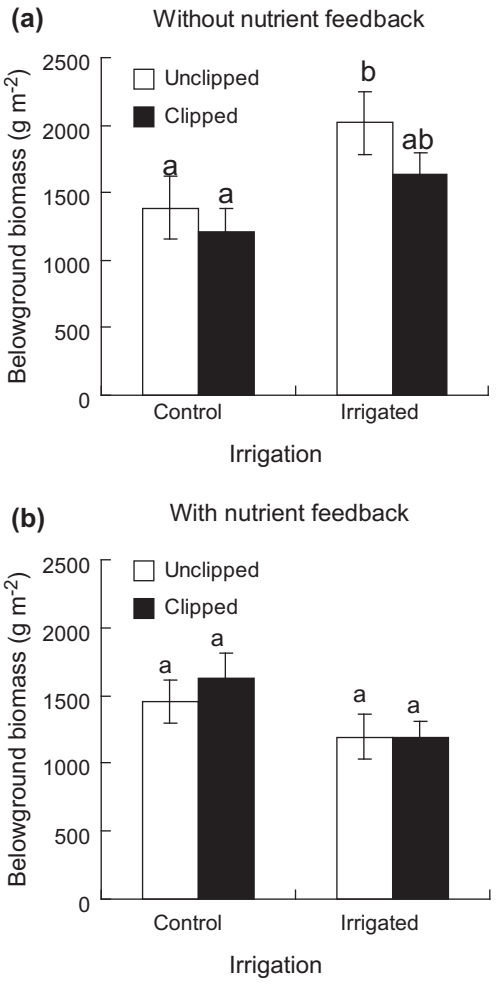

Figure 2. Mean (±1 SE) belowground biomass (g m^{-2}) of clipped and unclipped plots under two levels of water-availability (irrigated and non-irrigated). Results under the presence of nutrient-feedback pathways arising from dung-application (a) and in the absence of nutrient-feedbacks (b) are shown separately. Different letters show differences among means in a multiple-comparison test.

unclipped plots regardless of their fertilization status (Fig. 1). Further, in the unclipped plots, irrigation and fertilization together did not have synergistic effects on aboveground production (Fig. 1). So, in absence of clipping, plant production is favored by increased water availability, regardless of nutrient feedbacks, and water appears to be the primary limiting resource. This result is consistent with previous studies in this high-altitude eco-region where plant production was found most sensitive to changes in precipitation (Christensen et al. 2004). However, the evidence for water-limitation was not straightforward among the clipped plots, because there were interactive effects between irrigation and fertilization. When plants were clipped, irrigation increased aboveground production, but, only in the fertilized plots (Fig. 1). But when plants were clipped, irrigation also led to lower aboveground production in the unfertilized plots (Fig. 1). So, unlike the unclipped scenario where water seems to be the primary limiting factor, the patterns under clipping indicate that nutrient-limitation also becomes important.

Such pluralistic trends in aboveground production are not entirely consistent with the predictions of the CCH and GRH. The data adhere to the predictions of GRH (i.e. under- or equal-compensation in fertilized plots and over-compensation in unfertilized plots), but only in the absence of irrigation. The results from irrigated plots appear to deviate from the GRH because there was under-compensation in unfertilized plots and over-compensation in fertilized plots (Fig. 1). Likewise, the data adhere to the predictions of CCH (i.e. equal- or overcompensation under irrigation), but only when plots were fertilized simultaneously. The results from plots that were irrigated but not fertilized appear to deviate from the CCH, as there was evidence of under-compensation (Fig. 1). Thus, as highlighted in reviews (Hawkes and Sullivan 2001), neither model by itself can fully account for the relationships between herbivory-tolerance and resource conditions, even within the same dataset.

Table 3. Results of mixed-model ANOVA to test interactive effects of clipping, fertilization by dung-application, and irrigation on root:shoot ratio in Spiti region of northern India. Site was included as a random factor. Both clipping and fertilization reduced root:shoot ratios, and the interaction between fertilization and irrigation was evaluated with multiple-comparison of means.

Effect	DF	SS	MS	F	p
Site	8	5.95	0.74	3.10	0.006
Clipping	1	0.93	0.93	3.88	0.05
Fertilization	1	1.72	1.72	7.17	0.01
Irrigation	1	0.01	0.01	0.03	0.86
Clipping × Fertilization	1	0.45	0.45	1.86	0.17
Clipping × Irrigation	1	0.45	0.45	1.88	0.17
Fertilization × Irrigation	1	2.23	2.23	9.34	0.003
Three-way interaction	1	0.13	0.13	0.57	0.45
Error	56	13.43	0.24		
Total	71	25.31			

These divergent trends are however, partially consistent with the LRM which makes a wide variety of predictions (Banta et al. 2010). Clipping appears to strengthen nutrient-limitation since clipped plants failed to compensate unless they were fertilized and irrigated simultaneously (Fig. 1). Irrigation alone, even though water appears to be limiting production in absence of clipping (Fig. 1a–b), did not lead to equal- or over-compensation in aboveground production among the unfertilized plots. While the LRM framework recognizes that herbivory may affect a limiting resource or an alternative resource, such an apparent transition from water- to nutrient-limitation is not explicitly predicted by it (Wise and Abrahamson 2005). Our results suggest that this transition is more likely due to co-limitation under clipping – by both water and soil-N (Saito et al. 2008). The potential for such co-limitation may arise because plant uptake of water and N from soil, does not occur through mutually independent pathways (Everard et al. 2010).

In other words, unclipped plants appear to be primarily water-limited, but, clipping introduces stronger nutrient-limitation and establishes co-limited conditions. Importantly, compared to the respective controls, unclipped plants showed increased production when irrigated, regardless of fertilization. But, a corresponding response was not seen among the clipped plants since they increased production compared to respective controls only when fertilized and irrigated together. When clipped plots were irrigated, production decreased in unfertilized plots, whereas, production increased

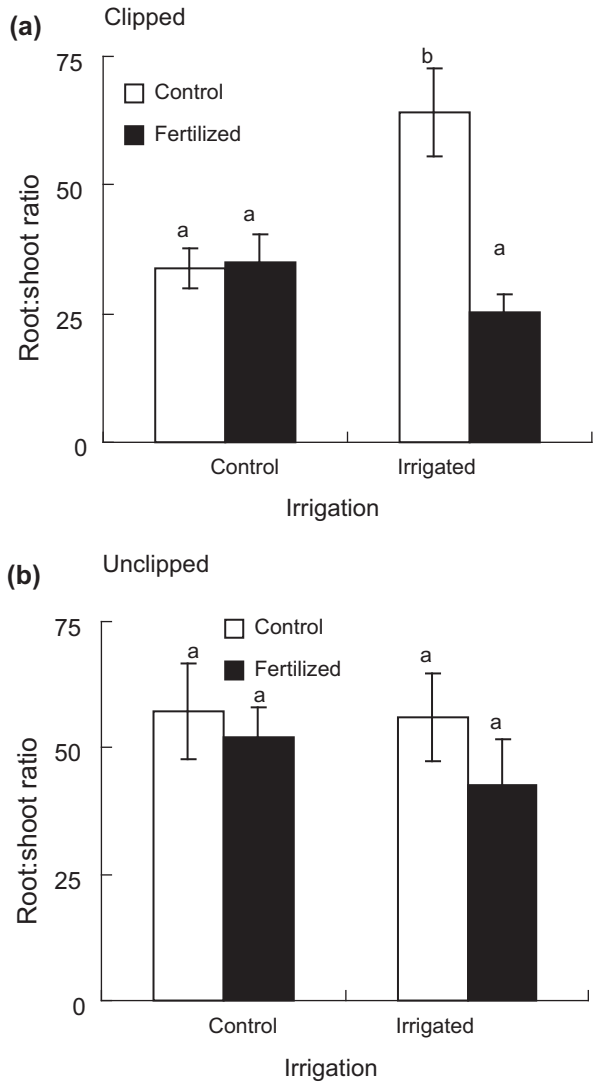

Figure 3. Mean (±1 SE) root:shoot ratio of fertilized and unfertilized plots under two levels of water-availability (irrigated and non-irrigated). Results for clipped (a) and unclipped plots (b) are shown separately. Multiple-comparison of means over the significant interaction between fertilization and irrigation (Table 3) shows that irrigation in absence of fertilization led to increased root:shoot ratios in clipped plots (a), but not for unclipped plots (b). Different letters indicate differences among means.

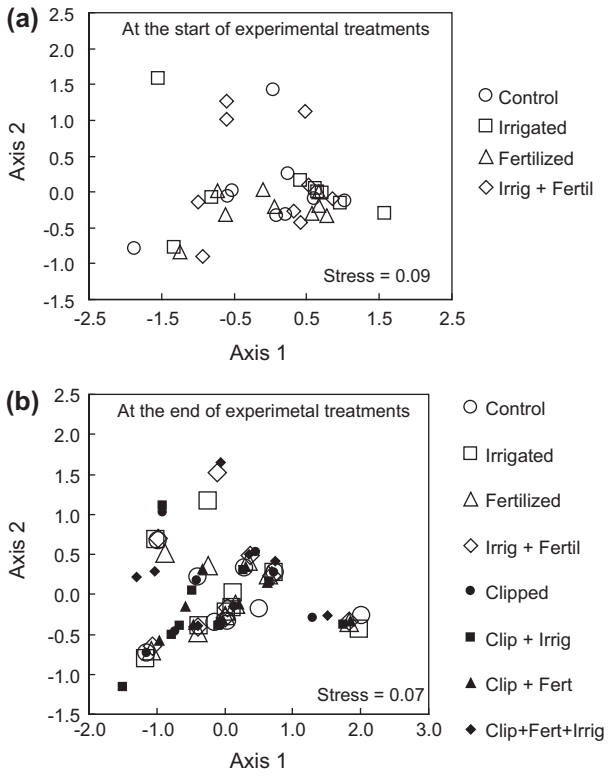

Figure 4. Nonmetric multidimensional scaling (MDS) over two axes of compositional similarity of vegetation sorted into four functional groups (grasses, sedges, forbs and shrubs) at the beginning of the study from the clipped plots (a) and at the end of the study from all plots (b). The MDS results show that while there were compositional differences among the plots, these did not vary systematically among the treatment conditions. So, it is unlikely that our results on plant tolerance (Fig. 1–2) are an artifact of compositional changes occurring in the plots.

in the fertilized plots (Fig. 1). Thus, unlike the unclipped plants which appear water-limited, clipped plants seem to be co-limited by water and nutrients as herbivory appears to increase the relative strength of nutrient-limitation. Therefore, these data appear to be only partially consistent with the predictions of the LRM. This may be because the LRM framework incorporates a relatively simplistic scenario of herbivore effects on a limiting or on an alternative resource (Wise and Abrahamson 2005). And since the latter is presumed to be non-limiting, production is expected to be unaffected by its availability (i.e. Liebig's law, De Baar 1994), as was evident from the difference in response of unclipped plants to irrigation and fertilization (Fig. 1). Although the LRM does attempt to encompass a scenario wherein herbivory may cause such a secondary alternative resource to become limiting, it does not provide a mechanistic explanation for such an outcome (Wise and Abrahamson 2005). Such a mechanism may involve the inter-related nature of water and N uptake pathways by roots (Everard et al. 2010).

Although the LRM seems relatively more successful at explaining our results than either the CCH or the GRH, it appears to be restricted in its scope to account for an apparent transition in the relative strengths of water- and N-limitation following herbivory. Specifically, the LRM does not completely explain the observed lower tolerance in irrigated plots in the absence of fertilization. This transitional trend, however, is consistent with reports from aquatic ecosystems where herbivory by zooplankton is seen to regulate the relative degree of N and phosphorus (P) limitation on phytoplankton (Elser et al. 1988). Comprehensive reviews of different datasets have found that such co-limitation is not only widespread in aquatic ecosystems, but also in terrestrial ecosystems (Elser et al. 2007). These insights indicate that plant growth conditions are often near the boundary where an alternative resource can become limiting, and herbivory can induce a transition between these states (Elser et al. 1988, 2007).

Similar to alteration of the relative strengths of N and P co-limitation in aquatic ecosystems (Elser et al. 1988), previous work has recognized that relative allocation to above- and belowground compartments may indicate such a transition between water and nutrient-limitation in

terrestrial ecosystems (Burke et al. 1998). If experimentally provisioning water alleviates water-limitation, then it can lead to stronger nutrient-limitation under herbivory (Burke et al. 1998). Under such conditions, classical theory suggests that plants should invest more in belowground production (Tilman 1988, Wilson and Tilman 1991, Canham et al. 1996, Cahill 1999). High root:shoot ratio across the greater Trans-Himalayan grazing ecosystem (Yang et al. 2010) indicate such a trend (Hutchings and John 2004). Our results from belowground biomass (Fig. 2) and root:shoot ratios (Fig. 3) are also consistent with this expected trend. The belowground data suggest that while irrigation led to increased belowground biomass in unfertilized plots, it had no effect under dung-application (Fig. 2). For root:shoot ratio, the significant interaction between fertilization and irrigation (Table 3) clarifies the response in relative biomass allocation. Among clipped plots, irrigation led to increased root:shoot ratio in absence of fertilization (Fig. 3a). But, the corresponding response was lacking among the unclipped plots (Fig. 3b). This provides qualitative support for the idea that as the over-riding importance of water as a limiting factor diminishes, nutrients such as soil-N can become progressively more important in determining plant production under herbivory (Burke et al. 1998), and this effect was more prominent under simulated herbivory. Interestingly, recent evidence also seems to indicate that the corollary – increased nutrient availability reciprocally strengthening water-limitation – can also occur (van der Waal et al. 2009), thus indicating the flexibility of the co-limitation framework to explain a wide variety of resource-dependent outcomes.

Arguably, an alternative explanation might be that our dung-application treatment was rather ineffective in establishing a positive nutrient feedback (equivalent to only 1.8 g m^{-2} of N). Oven-drying the dung might reduce its decomposability compared to natural dung. However, oven-drying at 40°C might not be severely damaging because, under normal field conditions, the exposed surfaces of dung-pellets may reach temperatures of 55–60°C (Bagchi unpubl.). Difference in micro-site conditions could introduce also some variation in the rate at which nutrients entered the soil-pool through decomposition. Yet, such an argument over ineffective fertilization effects of dung seems tenuous since either the main effect or the interactive effects of dung-application were statistically significant in all cases (Table 1–3), and corresponding effect-size was large (Cohen's $f = 0.76 \pm 0.07$, estimated from Table 1–3). Instead, these results likely conform to the anticipated trend that plant production in this arid eco-region is more sensitive to changes in water-availability than other factors (Christensen et al. 2004), and the interactive effects of fertilization become important under grazing.

The dichotomous CCH-GRH view appears to have important shortcomings in explaining highly variable responses in herbivory-tolerance even within the same ecosystem (Fig. 1). The more integrative LRM framework, which incorporates herbivore-mediated indirect nutrient-feedback pathways, holds relatively greater promise for a better understanding of the mechanisms that may determine the variation in herbivory-tolerance in plants. However, since the LRM framework does not yet include a mechanistic pathway for herbivore-mediated transitions between relative influences of potentially co-limiting resources, our results identify the scope for developing a more comprehensive theory based on co-limitation. Conceptually, nutrient co-limitation is believed to be an under-utilized, and perhaps even misunderstood, aspect of plant-herbivore interactions (Saito et al. 2008). To date, available theoretical models of plant production under co-limited conditions (Legovic and Cruzado 1997, Klausmeier et al. 2004) do not explicitly incorporate the role of herbivores. Likewise, the role of herbivory remains to be incorporated into theories of co-dependent pathways for plant uptake of water and soil-N (Everard et al. 2010). So, these models of multiple-resources do not generate predictions which can parallel those from more conventional plant-herbivore models (Loreau 1995, de Mazancourt et al. 1998, de Mazancourt and Loreau 2000). Yet, our data along with other empirical studies (Elser et al. 1988) indicate that herbivores may regulate the degree of limitation by multiple resources on plant production in aquatic as well as terrestrial ecosystems. Thus, it appears that a more comprehensive understanding of why herbivores have variable effects on plant production at different sites may be found in their influences on co-limiting resources, and this might be achieved by further refinements to the LRM framework.

Better conceptual frameworks for herbivory-tolerance may not only further our understanding, but may also have important applications for land-management (Levin 1993, Juenger and Lennartsson 2000), particularly in the wake of climate and land-use change scenarios. In the greater Trans-Himalayan ecosystem including the Tibetan highlands, there appears to be a trend towards increased summer precipitation since the 1980s (Tao et al. 2004, Shaohong et al. 2007). Yet, paradoxically, this coincides with accelerated vegetation degradation over the same time period which is not fully explained by changes in animal stocking densities alone (Ding et al. 2007). Our data can provide some insights into this apparently counterintuitive trend. Possibly, the observed vegetation degradation across large tracts of the Tibetan highlands is likely related to the alteration in relative strength of nutrient- and water-limitation as a result of altered precipitation and human use. Therefore, better informed management of human-mediated nutrient immobilization, and even loss, could be key for sustainable grazing in this ecosystem.

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