

Mechanisms linking plant diversity to large herbivore performance

Ling Wang^{1*}, Deli Wang^{1*}, Zhengbiao He¹, Guofang Liu¹ & Ken C. Hodgkinson²

¹*Institute of Grassland Science, Northeast Normal University, and Key Laboratory of Vegetation Ecology, Ministry of Education, Changchun, Jilin 130024, China.* ²*CSIRO Sustainable Ecosystems, GPO Box 284, Canberra, ACT 2601, Australia.*

**These authors contributed equally to this work.*

There is established concern that loss of biodiversity will affect ecosystem productivity, nutrient cycling, carbon storage, stability and other properties^{1,2}. Interactions between trophic levels are thought to link changes to biodiversity and ecosystem processes³⁻⁶. However, there is a lack of empirical studies linking plant diversity with altered trophic levels^{7,8}, especially for large herbivores, the important but often neglected, controlling trophic level in terrestrial systems. Here we examine responses in performance of the large generalist herbivore to changes in plant diversity, using an indoor cafeteria trial and a field experiment. Our results show that increased plant diversity improves herbivore performance but it is depressed at highest plant diversity levels. We propose the *Disturbance Selection Hypothesis* for explaining plant diversity effects on primary consumers. Increasing the number of plant species in grassland, increases consumption and enhances nutrient intake (presumably improving animal fitness) by modifying nutrient balance, toxin dilution and taste modulation. High plant diversity simultaneously intensifies animal diet switching frequency, and weakens the herbivore's ability to select food, thereby increasing foraging cost and disturbing the herbivore's selection of forage. Thus, the consequence of plant diversity for large herbivore performance depends on the trade-off between the positive and negative effects. At

highest plant diversity the positive effects weaken and negative effects strengthen. We suggest knowledge of the mechanisms is the means for understanding relationships between biodiversity and ecosystem functioning, and the management of large herbivores on rangelands used for conservation and grazing.

Changes in plant diversity, especially those influencing nutrient dynamics and trophic interactions, affect the structure and functioning of ecosystems⁹. Empirical and theoretical work on the functional consequences of changed plant diversity on ecosystem function have focused on functioning process within a single trophic level, such as primary productivity, community stability and nutrient utilization^{1,10,11}. Effects of changed plant diversity on adjacent trophic organisms, the primary consumers, which in turn directly affect secondary production, are poorly documented. Herbivores have strong impacts on ecosystem process by mediating energy transfer and nutrient cycling, and indirectly altering plant biomass, community composition and distribution¹². Effect of changing plant diversity on herbivore performance is therefore a key issue.

Plant diversity effects on small herbivores have been studied. Declining plant diversity reduces diversity of herbivorous insects^{7,13}, increases abundance of specialist insects^{14,15}, and decreases performance (biomass gain, survival rate and reproduction) of small generalist herbivores^{16,17}. Other experiments show that diversity and identity of plant functional groups influence herbivore abundance and performance^{8,18}. Theoretical interpretations of the response of specialist herbivores to plant diversity have been suggested^{19,20}. Surprisingly, little is known about plant diversity effects on the performance of large generalist herbivores. They strongly control many grassland ecosystem processes and impact on provision of ecosystem goods and service, and hence human survival²¹. Large herbivores (usually generalist), commonly cope with the low plant nutrient content of grassland forage but require higher abundance of energy-rich plants to maximize performance. Small herbivores, on the other hand, select nitrogen-rich foods and exhibit some degree of host plant specificity^{22,23}. There should

be different mechanisms of plant diversity impacts on performance of large compared with small herbivores. Here we tested nutritional and functional outcomes of increasing plant diversity for a large generalist herbivore, and simultaneously examined foraging behaviour responses to changing plant diversity, which provides mechanistic insight into the functional consequences of plant diversity.

Plant diversity changes influence food availability and quality for herbivores, which in turn affects herbivore foraging patterns for the nutrients required for survival, growth and reproduction. Energy and protein are critical. We specifically measured energy (Metabolism Energy, ME) and protein (Crude Protein, CP) intake, besides food consumption, in order to estimate animal performance. Because large herbivores, unlike small invertebrates, have long life spans and are difficult to study experimentally, we used energy/protein intake as a surrogate for performance²⁴.

Foraging is a very complex process, and herbivores make decisions on which plants to consume, with the outcomes influencing their nutrient acquisition. During foraging they may switch the type of plants eaten to maximize intake²⁵. Diet switching is a cost to herbivore performance. The quality of foods chosen by herbivores is termed 'diet selection ability'. Here we primarily examine change in diet switching frequency and diet selection ability of sheep to establish the mechanisms that link performance of large herbivore to altered plant diversity.

This study was conducted in a semi-controlled indoor cafeteria and in the field. The cafeteria experiment enabled elucidation of mechanisms that could not be studied in the field. In the cafeteria, we created six plant diversity levels with 1, 2, 4, 6, 8 and 11 species using an additive experimental approach, and at each level there were three species compositions for replication. Concurrently, a grazing experiment was conducted in the field utilizing plots at three diversity levels (1, 4-6, and >8 species).

We found the number of plant species eaten by sheep increased with plant diversity and sheep included all plant species in their diet (Fig. 1). There were different

proportions of the species eaten from each of the three compositions (Fig. 1). Sheep are selective and the amounts of particular species consumed depend strongly on the context in which the food is presented. Irrespective of the proportion of the presented diet, the consumptions significantly increased in all three combinations with increasing plant diversity ($F_{5, 10}=57.90$, $P<0.0001$; $F_{5, 10}=94.47$, $P<0.0001$; $F_{5, 10}=34.20$, $P<0.0001$, respectively; Fig. 1a, b, c).

Analyzing all combinations, we found a 2.4-fold increase in daily dry matter intake from one species in the diet to 11 species ($F_{5, 40}=108.27$, $P<0.0001$; Fig. 2a). There was satiation after 8 species. In the field there was also a significant increase in food intake of sheep grazing the more plant diversity communities ($P<0.05$; Fig. 2a'). The energy/protein ratio did not differ among 1, 2, 4, 6 and 11 species levels (Fig. 2b), but there was still a significant increase in food intake (Fig. 2a), probably because of taste modulation or toxin dilution. Although we can not preclude a toxin dilution effect, this study does show that taste modulation plays a more important role in herbivore foraging than previously thought²⁶.

Nutrient intake is improved by greater plant diversity ($F_{5, 40}=78.70$, $P<0.0001$ for ME intake; $F_{5, 40}=57.12$, $P<0.0001$ for CP intake; Fig. 2c, d). However, metabolism energy intake slowed above 8 species (Fig. 2c) and crude protein intake did not increase above 4 species (Fig. 2d). Overall, the highest plant diversity offered to the sheep did not maximize nutritional benefits for this herbivore. At one species of plant, the amounts of energy and protein in the diet did not meet maintenance requirements.

To better understand the consequences of plant diversity for nutrient intake, we analyzed the quality of the diet. Trends in various nutrient concentrations (ME, CP, Neutral Detergent Fibre (NDF), and Acid Detergent Fibre (ADF)) were the same. The best diet quality was at intermediate levels of plant diversity not at highest levels (Fig. 3a-d). Hence, we conclude that the drop of nutrient intake at the highest diversity is partly attributed to lowered diet quality arising from a weakened diet selection ability.

Furthermore, our data showed that the sheep switched their feeding more frequently as the plant diversity rose (Fig. 3e). This switching inevitably increased the cost of foraging. For these reasons, we view the changed sheep foraging behaviour at higher plant diversity to be a disturbed foraging pattern.

In summary, it is clear that herbivores have a greater chance of selecting preferred food and to forage for a diverse diet, as the plant diversity increases, which positively affects the herbivore's consumption of food and enhances nutrient intake by modifying nutrient balance²⁷, toxin dilution²⁸ and taste modulation etc. (Fig. 4a). These positive effects of plant diversity have also been observed in small generalist herbivores, but there are different operating mechanisms. In our study, the benefits of diverse plant resources for large herbivores came not only from higher food quality, but also greater food consumption. In contrast, for small herbivores the benefits of diverse plant resources only come from the high quality of plants available for consumption¹⁶. Although higher plant diversity increases the options available, too much choice confounds herbivore discrimination and increases the cost for foraging leading to lowered ability to select the best foods and obtain greatest benefit (Fig. 4a). We explicitly showed that the quality of the diet of the sheep was not best at the highest plant diversity, and the energy costs for diet switching increased significantly. Taken together, the functional consequence of plant diversity for large herbivores depends on a trade-off between selecting for diet quality and the consequences of higher energy costs.

We concluded that the hypothetical model for functional consequences of changing plant diversity can be divided into three phases (Fig. 4b). At lower diversity levels, large herbivores can not satisfy minimum nutrient requirements for maintenance and we call this the 'constraint phase'. As plant diversity increases, herbivores have more available resources and opportunities to select food which provides the nutrition for greater performance requirements and in this phase herbivores constantly regulate their diet to reach optimal condition (termed the 'optimizing phase'). Further increase in plant

diversity disturbs herbivore foraging, thereby lowering nutrient intake (termed the ‘disturbed phase’). We think it unlikely that greater plant diversity than studied would further depress performance because of the positive effects of plant diversity on performance.

Several hypotheses about the generally functional role of species diversity in ecosystems have been contextually developed, including the *diversity-stability*, the *rivet*, and the *redundancy* hypotheses, where the positive linear and asymptotic relationships respectively between diversity and rates of ecosystem processes have been presumed^{29,30}. There has been controversy over whether these conceptual hypotheses are universal. Our results suggest the diversity/productivity relationship may need revision and refinement on the basis that high diversity not only contains redundant elements but also causes disturbance to foraging. Consequently, we propose an alternative hypothesis, the *Disturbance Selection* Hypothesis, as a better elucidation for the functional consequences of plant diversity on large herbivore performance. Furthermore, high plant diversity in the generally low-quality grasslands is important for increasing consumption thereby promoting herbivore performance. Contrary to common belief, both outcomes are of significance to the conservation of plant species and to rangeland animal production.

Methods

Indoor cafeteria trial

Herbivore and plant species. Nine 2-year old male sheep (35.4 ± 1.8 kg) bred in northeast China were the herbivores. They are abundant throughout the region. Thirteen native plant species from three plant functional groups (grasses: *Leymus chinensis* (Trin.) Tzvel., *Phragmites australis* (Clav.) Trin., *Chloris virgata* Sw., *Hemarthria sibirica* (Gand.) Ohwi, *Calamagrostis epigejo* (L.) Roth., and *Echinochloa crusgalli* (L.) Beauv.; legumes: *Lathyrus quinquenervius* (Miq.) Litv. and *Vicia amoena* Fisch.;

forbs: *Kalimeris integrifolia* Turcz., *Artemisia scoparia* Waldstem et Kitailael, *Kochia sieversiana* (Pall.) C. A. M., *Apocynum venetum* L. and *Suaeda glauca* Bunge) selected as the trial plants, are common in the meadow steppes of northeast China. *Leymus chinensis* and *P. australis* are the dominant grass species locally.

Experimental design. The experiment was carried out in July/August 2005. Plants were collected from adjacent grassland every two days and stored in a cool room at 10°C. Sheep were individually housed in 2.5 m × 3.0 m pens two weeks before the experiment. They had free access to water and were offered fresh plants twice daily at approximately 0730 and 1430 hours. Meals were removed after two hours. Plant species was separately presented in containers placed close together. Containers were positioned sequentially each day to ensure that ingestion was not influenced by the order of containers. More than enough plant material was added to each container for the 2 hour meal. Containers of each plant species were weighed before and after each meal. Water content of each plant species was measured before and after each meal and a correction was made for water loss. Behavioural data for all nine sheep were separately measured by nine observers. The foraging processes were determined from recordings of foraging time for each species. The number of diet switches during each meal was counted.

The experimental design was a randomized block design with six species levels and nine blocks. Plant species levels were 1, 2, 4, 6, 8 and 11 species designed by an additive approach (consecutively adding new plant species on the basis of the original species). Within each diversity level, there were three different species compositions. Species are grouped according to functional type (see Supplementary Information). The most abundant and dominant plant species from each of the 3 functional groups in the grassland were the first to be added. Overall, there were 9 sheep replicates for each level of plant diversity. Each level was offered for eight consecutive days, with statistical analysis performed on data averaged for the eight days.

Sampling and measurement. Samples of each species were separately collected daily and pooled over the eight days for each diversity level. Dried and ground samples were analyzed for CP, NDF, ADF and ME (see Supplementary Information).

Field experiment

The field study was conducted at the Songnen Grassland Ecological Research Station (44°45'N; 123°45'E), Northeast Normal University, Changling County, China. Three plant diversity levels with 1, 4-6 or >8 species, each with six replicate fenced plots (each about 200 m²), were selected in grassland utilizing the spatial diversity between 15 July and 20 August 2005. One species plots area dominated by either *Le. chinensis*, *P. australis* or *C. virgata*, were selected, fenced and weeded. Plots with 4-6 species comprising *Le. chinensis*, *P. australis*, *Ka. integrifolia*, *A. scoparia*, *Puccinellia tenuiflora*, *Thalictrum simplex*, *Ko. sieversiana* and *La. quinquenervius* were selected and weeded within the grassland. Plots with >8 species were selected and contained these additive species, *Arundinella anomala*, *Inula japonica* and *Taraxacum sinicum*.

Five adult male sheep (60.7 ± 5.5 kg) were chosen to graze for one day from 0700 to 0900 hours and from 1530 to 1730 hours at each plot. To estimate intake, three sheep were observed and total foraging bites were measured. Bite size was the average dry matter of one bite foraged by sheep, and estimated by measuring the amount of biomass eaten during foraging and divided by the number of bites. Intake mass was calculated using bite number multiplied by bite size. Sheep were fed by equally supplementary corn (400 g/sheep) in doors in each evening.

Statistical analysis

Statistical analyses were performed with the SAS 6.12 statistical package (SAS Institute Inc. 1989). For the indoor cafeteria trials, the daily dry matter intake, nutrient intake and behavioural measures were averaged over 8 days for the nine sheep and analyzed by

two-way ANOVA. Statistical difference between levels was determined by Duncan's tests, with $P < 0.05$ indicating significance. Because Levene's test confirmed that variances were not homogeneous across diversity treatments for intake data in field experiments, and diet quality (CP, ME, NDF and ADF concentrations) data and energy/protein ratio data in indoor cafeteria trials, and variances were still not homogeneous after data were further square-root and natural-logarithm transformed, these data were analyzed using the non-parameter test of Kruskal-Wallis.

1. Tilman, D. *et al.* The influence of functional diversity and composition on ecosystem processes. *Science* **277**, 1300–1302 (1997).
2. Hooper, D.U. *et al.* Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. *Ecol. Monogr.* **75**, 3–35 (2005).
3. Worm, B. & Duffy, J.E. Biodiversity, productivity and stability in real food webs. *Trends Ecol. Evol.* **18**, 628–632 (2003).
4. Thébault, E. & Loreau, M. Food-web constraints on biodiversity-ecosystem functioning relationships. *Proc. Natl Acad. Sci. USA* **100**, 14949–14954 (2003).
5. Downing, A.L. & Leibold, M.A. Ecosystem consequences of species richness and composition in pond food webs. *Nature* **416**, 837–841 (2002).
6. Cardinale, B.J. *et al.* Effects of biodiversity on the functioning of trophic groups and ecosystems. *Nature* **443**, 989–992 (2006).
7. Siemann, E., Tilman, D., Haarstad, J. & Ritchie, M. Experimental tests of the dependence of arthropod diversity on plant diversity. *Am. Nat.* **152**, 738–750 (1998).
8. Scherber, C. *et al.* Effects of plant diversity on invertebrate herbivory in experimental grassland. *Oecologia* **147**, 489–500 (2006).
9. Chapin III, F.S. *et al.* Consequences of changing biodiversity. *Nature* **405**, 234–242 (2000).
10. Hector, A. *et al.* Plant diversity and productivity experiments in European

- grasslands. *Science* **286**, 1123–1127 (1999).
11. Spehn, E.M. *et al.* Ecosystem effects of biodiversity manipulations in European grasslands. *Ecol. Monogr.* **75**, 37–63 (2005).
 12. McNaughton, S. J., Banyikwa, F. F. & McNaughton, M. M. Promotion of the cycling of diet-enhancing nutrients by African grazers. *Science* **278**, 1798–1800 (1997).
 13. Knops, J. *et al.* Effects of plant species on invasion dynamics, disease outbreaks, insect abundances and diversity. *Ecol. Lett.* **2**, 286–293 (1999).
 14. Koricheva, J., Mulder, C.P.H., Schmid, B., Joshi, J. & Huss-Danell, K. Numerical responses of different trophic groups of invertebrates to manipulations of plant diversity in grasslands. *Oecologia* **125**, 271–282 (2000).
 15. Andow, D.A. Population dynamics of an insect herbivore in simple and diverse habitats. *Ecology* **71**, 1006–1017 (1990).
 16. Pfisterer, A.B., Diemer, M. & Schmid, B. Dietary shift and lowered biomass gain of a generalist herbivore in species-poor experimental plant communities. *Oecologia* **135**, 234–241 (2003).
 17. Giulio, M.D. & Edwards, P.J. The influence of host plant diversity and food quality on larval survival of plant feeding heteropteran bugs. *Ecol. Entomol.* **28**, 51–57 (2003).
 18. Haddad, N.M., Tilman, D., Haarstad, J., Ritchie, M. & Knops, J.M.H. Contrasting effects of plant richness and composition on insect communities: a field experiment. *Am. Nat.* **158**, 17–35 (2001).
 19. Root, R.B. Organization of plant-arthropod association in simple and diverse habitats: the fauna of collards (I. *Brassica oleracea*). *Ecol. Monogr.* **43**, 95–124 (1973).
 20. Otway, S.J., Hector, A. & Lawton, J.H. Resource dilution effects on specialist insect herbivores in a grassland biodiversity experiment. *J. Anim. Ecol.* **74**, 234–

- 240 (2005).
21. Owen-Smith, N. *Megaherbivores*. The influence of very large body size on ecology (Cambridge University Press, Cambridge, 1988).
 22. Stephens, D.W. & Krebs, J.R. *Foraging Theory*. The economics of choice: trade-offs and herbivory. (Princeton University Press, New Jersey, 1986).
 23. Olf, H., Ritchie, M.E. & Prins, H.H.T. Global environmental controls of diversity in large herbivores. *Nature* **415**, 901–904 (2002).
 24. Simpson, S.J., Sibly, R.M., Lee, K.P., Behmer, S.T. & Raubenheimer, D. Optimal foraging when regulating intake of multiple nutrients. *Anim. Behav.* **68**, 1299–1311 (2004).
 25. Wiggins, N.L., McArthur, C. & Davies, N.W. Diet switching in a generalist mammalian folivore: fundamental to maximizing intake. *Oecologia* **147**, 650–657 (2006).
 26. Marsh, K.J., Wallis, I.R., McLean, S., Sorensen, J.S., & Foley, W.J. Conflicting demands on detoxification pathways influence how common brushtail possums choose their diets. *Ecology* **87**, 2103–2112 (2006).
 27. Westoby, M. What are the biological bases of varied diets? *Am. Nat.* **112**, 627–631 (1978).
 28. Freeland, W.J., & Janzen, D.H. Strategies in herbivory by mammals: the role of plant secondary compounds. *Am. Nat.* **108**, 269–289 (1974).
 29. MacArthur, R. Fluctuations of animal populations and a measure of community stability. *Ecology* **36**, 533–536 (1955).
 30. Walker, B. Biodiversity and ecological redundancy. *Conserv. Biol.* **6**, 18–23 (1992).

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Author Contributions D. W. envisioned the experiment. L. W. performed the main experiment, and Z. H. and G. L. performed a part of the experiment. L. W. and D. W. discussed the results, developed, and wrote the paper. K. C. H. commented on the science and revised the manuscript.

Author Information Correspondence and requests for materials should be addressed to D. W. (wangd@nenu.edu.cn, wangd737@nenu.edu.cn).

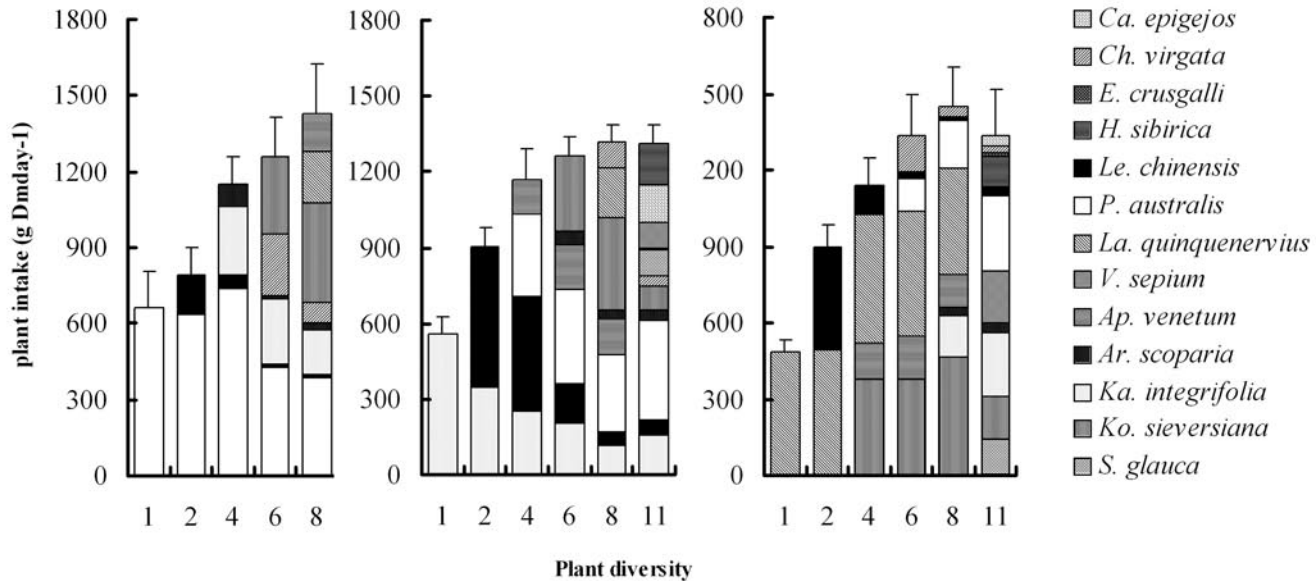
Figure 1 Effects of plant diversity on daily food intake of sheep for three plant species compositions (**a**, **b**, and **c**). The histograms represent the average mass of each plant species eaten. Bars are standard errors for total intake of all plants.

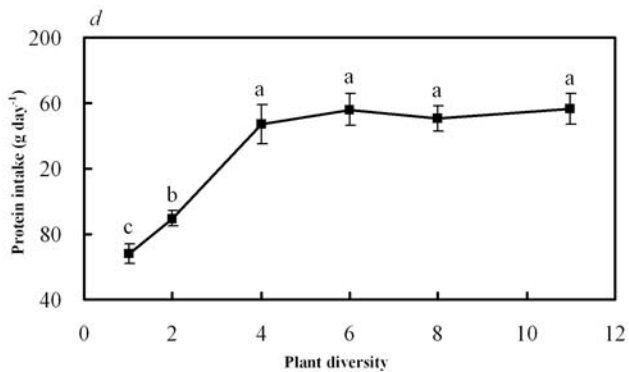
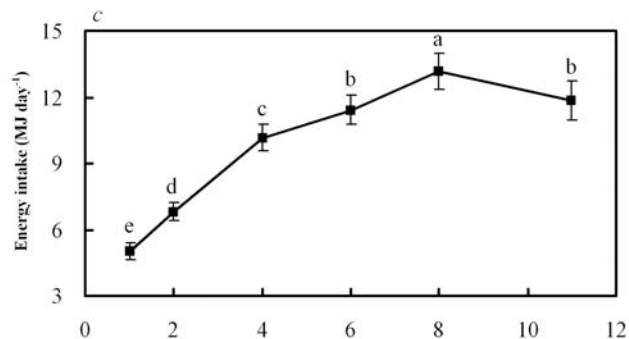
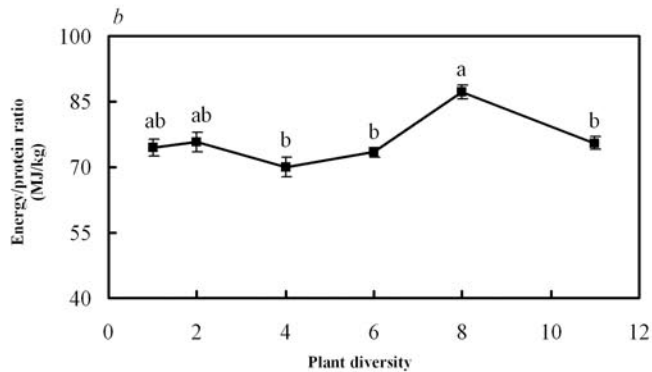
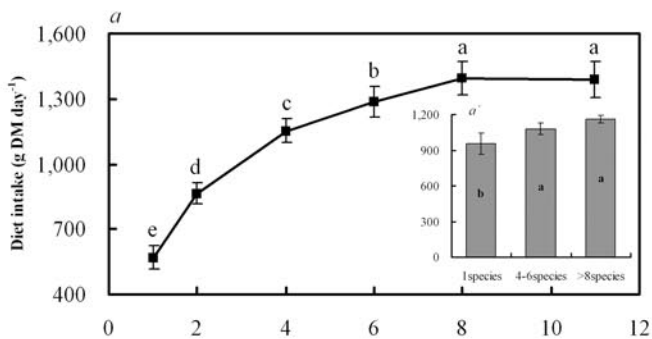
Figure 2 Effects of plant diversity on the diet of sheep. **a**, the average daily dry matter (DM) intake (**a'**, the average daily DM intake in field grazing experiment). **b**, energy/protein ratio. **c**, the average daily ME intake. **d**, the average daily protein intake. Points are the means for nine sheep measured over 8 days and with three species compositions within each level. Different letters indicate points significantly different from each other ($P < 0.05$).

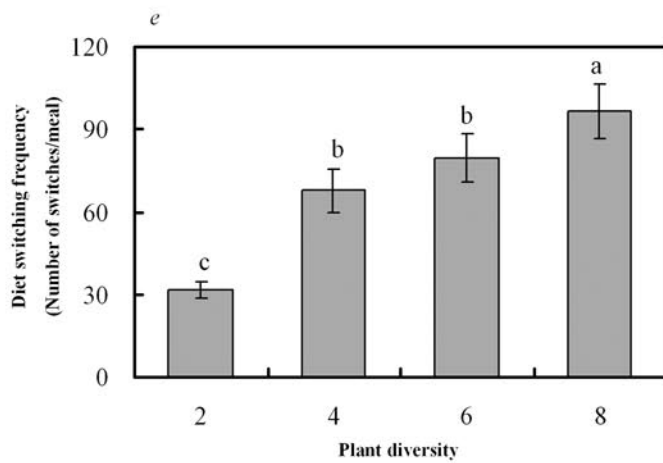
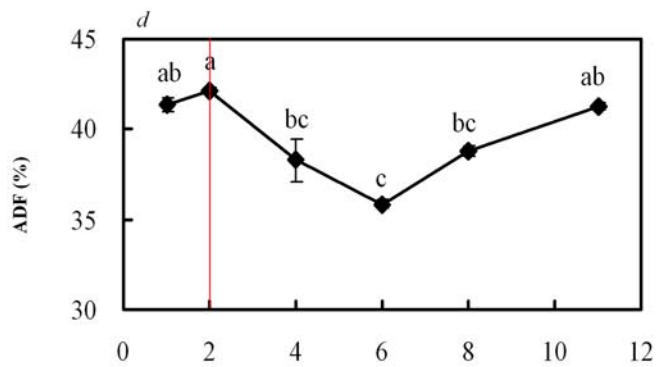
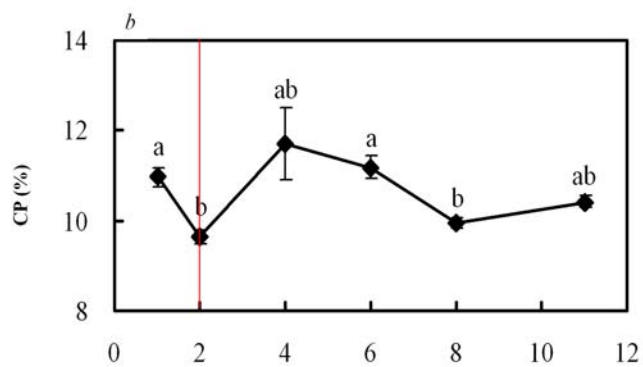
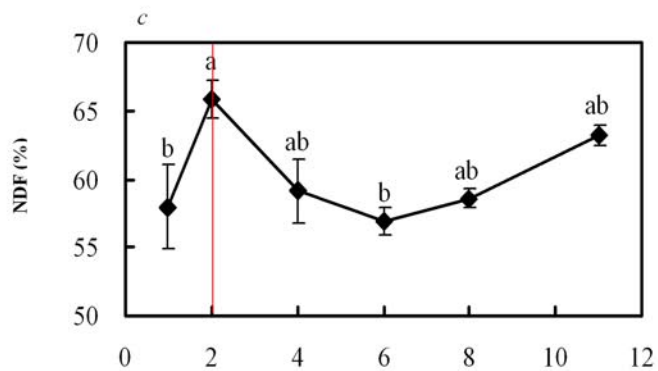
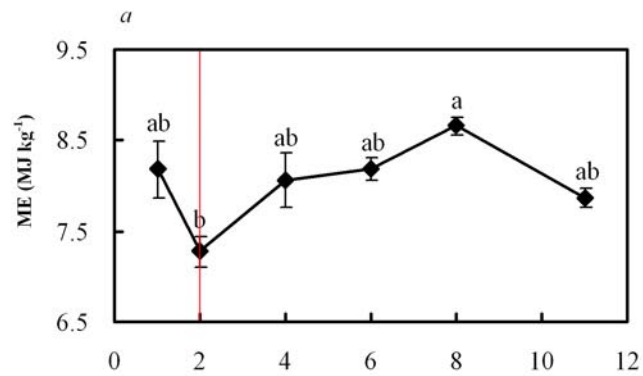
Figure 3 Effects of plant diversity on sheep diet selection ability (diet quality) and diet switching frequency. **a-d**, Diet nutrient concentrations were calculated from average daily nutrient intake divided by average daily dry matter intake. Points are the means for nine sheep measured for three species compositions within each level. On the left of the vertical line, animal can not exhibit selectivity for plants because only one species is available; On the right, the nutrient concentrations in the diet indicates the magnitude of selection ability by sheep.

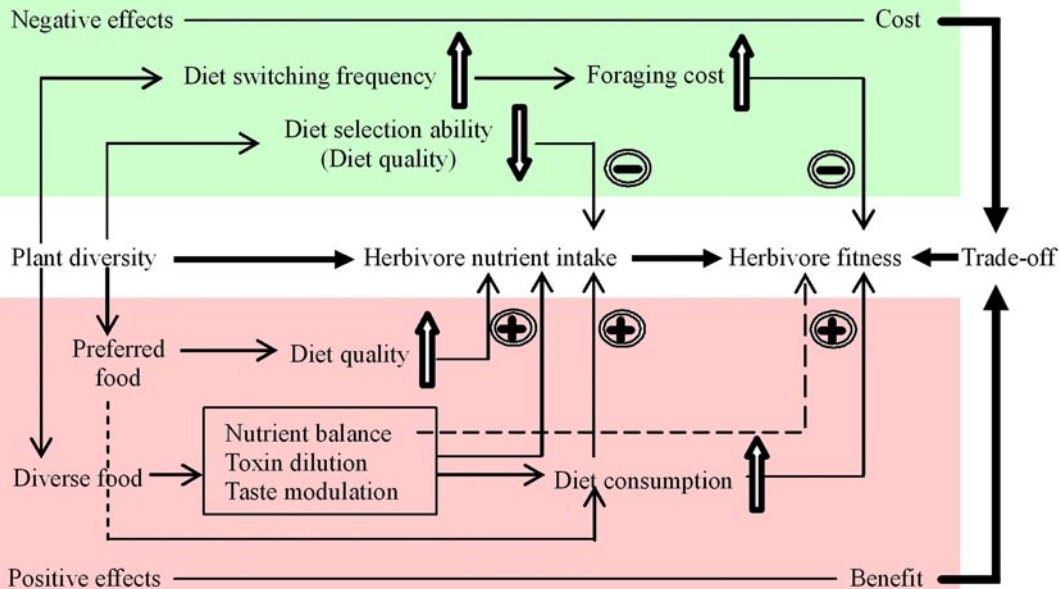
ME (**a**), CP (**b**), NDF (**c**), ADF (**d**). **e**, Diet switching frequency within each meal averaged over eight days. Points with different letters are significantly different from each other ($P < 0.05$).

Figure 4 Mechanisms and hypothetic model of functional consequences of plant diversity to large generalist herbivore. **a**, Mechanisms by which plant diversity affects performance of large generalist herbivores. Up-arrows indicate an increase, and down-arrows a decrease. Plus signs are a positive effect, and minus signs a negative effect. **b**, Hypothetic model of consequences of plant diversity for performance of large generalist herbivores. The dotted line indicates threshold of maintenance requirements for herbivores; above the threshold the requirement is met, below it is not. The two vertical lines divide the response into three phases: constraint, optimizing and disturbed.







a*b*