

Journal of Ecology

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Article type : Research Article

Editor : Carla Staver

Herbivore dung stoichiometry drives competition between savanna trees and grasses

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Abstract

1. The balance between trees and grasses is a key aspect of savanna ecosystem functioning, and so far, believed to be regulated by resource availability, fire frequency and consumption by mammalian herbivores. Herbivores, however, also impact plant communities through the deposition of growth-limiting nutrients in their dung and urine. Little attention has been paid to the fact that savanna herbivores produce dung containing different concentrations of nutrients and it remains unknown what the effect of this variation is on tree-grass interactions.
2. Here, we investigate if stoichiometric differences in dung from browsers and grazers from an African savanna are large enough to influence competitive interactions between N₂-fixing trees and grasses. We performed a competition experiment with seedlings of three common N₂-fixing tree species and three common C₄-grass species from a Kenyan savanna. Plants were grown in mesocosms as monocultures or mixtures with dung from either zebra (grazer) or giraffe (browser).

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version of Record](#). Please cite this article as doi: [10.1111/1365-2745.13623](https://doi.org/10.1111/1365-2745.13623)

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3. We show that variation in dung nitrogen (N) to phosphorus (P) ratio between these herbivores was large enough to drive the competitive outcome between tree seedlings and grasses. Under zebra dung with a low N:P ratio (3.5), tree seedlings had an advantage due to increased nodulation and N₂-fixation. Under giraffe dung with a high N:P ratio (6.4), grasses suppressed nodulation and tree seedling growth.

4. *Synthesis.* We identify another potentially important mechanism by which mammalian herbivores can stimulate ecosystem stability, namely through the N:P ratio of their dung. This illustrates how connected browsing and grazing herbivores are in African savannas, through the forage quality they consume, the N:P stoichiometry of their dung, and subsequent effects on plant competition.

Keywords: African herbivores, browser, faeces, grazer, nitrogen fixation, nutrient limitation, phosphorus, tree-grass balance

Introduction

Savanna ecosystems are characterized by a continuous grass layer with more or less densely scattered trees (Scholes & Archer, 1997), many with the ability to symbiotically fix atmospheric nitrogen (Högberg, 1986; Pellegrini, Staver, Hedin, Charles-Dominique, & Tourgee, 2016). The balance between the dominance of trees or grasses is ascribed to several factors including variation in resource availability (water and/or nutrients), fire frequency and activities of mammalian herbivores (Sankaran et al., 2005; Sankaran, Ratnam, & Hanan, 2008; Scholes & Archer, 1997; van Langevelde et al., 2003). The consumptive effect of herbivores on productivity of trees and grasses in savannas is relatively well-studied. Grazing herbivores have been shown to promote tree cover by reducing competition with grasses and reducing fuel loads for fires (Archibald, Bond, Stock, & Fairbanks, 2005; Kimuyu, Sensenig, Riginos, Veblen, & Young, 2014; Liedloff, Coughenour, Ludwig, & Dyer, 2001; Riginos & Young, 2007; Roques, O'Connor, & Watkinson, 2001; van Langevelde et al., 2003), thereby increasing tree growth and survival. Conversely, browsing herbivores can either promote tree cover by dispersing tree seeds and increasing their germination through digestion, or suppress it by browsing on trees and limiting their seed production and establishment (Barnes, 2001; Goheen, Palmer, Keesing, Riginos, &

Young, 2010; Staver & Bond, 2014; Young & Augustine, 2007). Herbivores, however, do not only impact plant communities through plant consumption, they also do so through the deposition of growth-limiting nutrients in their dung and urine (Bardgett & Wardle, 2003; Hobbs, 1996). So far, little attention has been paid to the fact that savanna herbivores produce dung containing different concentrations of nutrients (le Roux, van Veenhuisen, Kerley, & Cromsigt, 2020; Sitters, Maechler, Edwards, Suter, & Olde Venterink, 2014; Sitters & Olde Venterink, 2021a) and it remains unknown what the effect of this variation is on tree-grass interactions.

Besides water, plant productivity in savanna ecosystems is commonly limited by nitrogen (N) and/or phosphorus (P) (Augustine, McNaughton, & Frank, 2003; Cech, Kuster, Edwards, & Olde Venterink, 2008; Pellegrini, 2016) and herbivores are able to change the relative availabilities of these nutrients through their dung. Dung from mammalian herbivores species varies considerably in N:P stoichiometry, which can be linked to differences in herbivore size and diet (e.g. browsers vs. grazers) and thus the quality of plants consumed (le Roux et al., 2020; Sitters et al., 2014; Sitters & Olde Venterink, 2018, 2021a; Valdés-Correcher, Sitters, Wassen, Brion, & Olde Venterink, 2019). The variation in dung N:P stoichiometry in turn influences decomposition and the return rates of N and P to the soil, with lower relative losses of the least abundant nutrient (Sitters et al., 2014). Dung with a high N:P ratio is therefore likely to increase relative N availability more than P availability. As plants have certain N:P requirements for their growth, and these requirements differ largely among species (Fujita et al., 2014; Olde Venterink & Güsewell, 2010), this might change competitive interactions between plant species.

Indeed, in a previous study we showed that herbivore dung stoichiometry influenced the diversity and composition of an experimental plant community (Valdés-Correcher et al., 2019). Dung that provided the highest N:P ratio (in this case rabbit dung with N:P of 17) maintained the most diverse plant community, while dung with lower N:P ratios stimulated communities with higher abundances of leguminous forbs. The dominance of legumes was likely due to their ability to fix N₂, which under low N:P supply was not constrained by P availability (Augusto, Delerue, Gallet-Budynek, & Achat, 2013; Batterman, Wurzburger, & Hedin, 2013; Varma, Catherin, & Sankaran, 2018; Vitousek et al., 2002). Grasses grew best when supplied with high amounts of dung with higher N:P ratios. With their dense systems of fine roots, grasses are better competitors for both soil N and P than legumes, and grasses might also have higher plant N and P

use efficiencies (Cech, Edwards, & Olde Venterink, 2010; Craine, 2006; Tjoelker, Craine, Wedin, Reich, & Tilman, 2005). This works in the advantage of grasses under conditions of high soil N and P availabilities or under P-limited conditions (high N:P ratio). However, under N-limited conditions, grasses are not able to make use of the available P as they are constrained by low N, while legumes are not because of their ability to fix N₂. This study showed that both variation in nutrient quantity and quality of natural organic fertilizers (herbivore dung) are important in controlling the diversity of plant communities.

The aim of the present study was to investigate if differences in dung N:P ratio among browsers and grazers from an African savanna ecosystem are large enough to influence competitive interactions between N₂-fixing trees and grasses. Hence, we experimentally tested our hypothesized concept of savanna ecosystem functioning in which N₂-fixing trees and grasses, as well as the herbivores that feed on them, occupy opposite positions in a connected cycle of processes (Sitters & Olde Venterink 2018; Fig. 1a). If supported, this concept implies that the dung of herbivores promotes the growth of the plant type that is not their primary forage, i.e., browser dung promotes grass growth while grazer dung promotes tree growth. Browsers, such as giraffe, produce dung with higher N:P ratios (5.0 ± 0.4) than grazers (3.7 ± 0.5), such as zebra, which is related to the differences in N:P ratio of their forage (Fig. 1b). We question if this relatively small – albeit significant – difference in dung N:P ratio is large enough to induce differences in competitive abilities between tree seedlings and grasses. If so, the N:P ratio of their dung might be another potentially important mechanism by which browsers and grazers maintain tree-grass coexistence in savannas.

We therefore hypothesize that N₂-fixing tree seedlings will perform better under low N:P supply provided by dung from grazers, while grasses will perform better under high N:P supply with dung from browsers. We also predict that the competitive strength of N₂-fixing tree seedlings will at first increase with dung amount, because their ability to symbiotically fix atmospheric N₂ is constrained by the availability of P (Fig. 1c; Augusto et al., 2013; Batterman et al., 2013; Pons et al., 2007; Varma et al., 2018; Vitousek et al., 2002). As dung amount increases so does the availability of P and the level of N₂-fixation, giving tree seedlings a competitive edge over C₄-grasses, which are still experiencing N limitation. This is especially true under low N:P supply provided by dung from grazers. However, when dung amounts become too high,

increased N supply suppresses N₂-fixation (Barron, Purves, & Hedin, 2010; Batterman et al., 2013; Finzi & Rodgers, 2009; Pons et al., 2007; Varma et al., 2018) and tree seedlings lose their competitive edge to grasses, which are the better competitors for soil N and P (Fig. 1c; Tjoelker et al., 2005). Grasses will therefore perform better with higher dung amounts, especially under high N:P supply provided by dung from browsers (Fig. 1d). Here, we provide support for our conceptual model by presenting results from a competition experiment with seedlings of three common N₂-fixing tree species and three common C₄-grass species from a Kenyan savanna. Plants were grown in mesocosms as monocultures or mixtures with dung from either zebra (low N:P ratio) or giraffe (high N:P ratio) at three different amounts.

Material and Methods

Seed, dung and soil collection

We collected seedpods of three *Vachellia* (previous *Acacia*) tree species (*Vachellia drepanolobium*, *V. etbaica* and *V. gerrardii*) and seeds from three common C₄-grass species (*Bothriochloa bladhii*, *Pennisetum mezanum* and *P. stramineum*) in a semiarid savanna at Mpala Research Centre (36°52'E, 0°17'N) in Kenya in July 2015. Per species, seeds were collected from 10-20 individual trees or 20-30 grasses. Seeds were transported to the Vrije Universiteit Brussel (VUB) in Belgium and stored until needed. In January 2016, we collected fresh dung of at least five individuals of giraffe (*Giraffa camelopardalis*) and Burchell's zebra (*Equus burchelli*) in the same savanna. Dung samples were pooled per herbivore species, dried in the sun and transported to the VUB (ca. 3 kg of dung). Dung was ground with a Retsch Mixer Mill MM300 (Westburg). Total N concentrations in dung were analysed with an elemental analyser (Thermo EA Flash 1112). Total P concentrations were measured with a continuous segmented flow analyser (QuAAstro, SEAL Analytical), after combustion and hot HCl extraction (Andersen, 1976; Johengen, 1997). We also collected soil (ca. 5 kg) from under an *V. etbaica* tree in January 2016. It was sieved (2 mm) and the fresh soil was sealed and transported to the VUB where it was stored until needed. Ca. 10 g of this fresh soil was added to each mesocosm to provide soil microbes for dung decomposition and plant-microbe interactions (e.g., *Rhizobia* and mycorrhiza).

Tree-grass competition experiment

Mid-November 2016, we sowed the tree and grass seeds in germination trays in the greenhouse at the VUB. To increase germination rates, we soaked grass seeds in gibberellic acid-3 (50 µM) overnight (Ma et al., 2018). Tree seeds were scarified by removing a small section of the seed coat (Rugemalila, Morrison, Anderson, & Holdo, 2017) and soaked for 4 hours in lukewarm water. On 7-10 January 2017, seedlings were planted in 3L mesocosms filled with quartz sand and placed on containers to avoid free draining. Total N or P contents of the sand were below detection limits. Plants were grown in monocultures (i.e., two grass or two tree seedlings of the same species per mesocosm) or in mixtures (i.e., one grass and one tree seedling per mesocosm). The aboveground length of each seedling was measured and used to estimate seedling biomass at the start of the experiment using allometric relationships per plant species (see Appendix S1 in Supporting Information). The estimated seedling biomass (means \pm SE) at the start of the experiment was: 138 ± 3.6 mg for *V. drepanolobium*, 96 ± 3.0 mg for *V. etbaica*, 78 ± 2.0 mg for *V. gerrardii*, 53 ± 1.6 mg for *B. bladhii*, 12 ± 0.4 mg for *P. mezanum* and 35 ± 1.2 mg for *P. stramineum*.

A mesocosm received 2, 10 or 50 mg N in the form of giraffe dung (N:P ratio of 6.4 ± 0.4 ; n = 6) or zebra dung (N:P ratio of 3.5 ± 0.2 ; n = 6) (Table S1). These dung supply levels were based on results from previous dung experiments (e.g., Along, 2017; Valdés-Correcher et al., 2019) and were comparable to field conditions (e.g., N pool from herbivore dung ranges from 0.04 to 1.7 g m⁻² in several African savannas, while our supply levels ranged from 0.06 to 1.5 g m⁻²; Augustine et al., 2003; Cech, Olde Venterink, & Edwards, 2010; Fornara & du Toit, 2008; Sitters, Kimuyu, Young, Claeys, & Olde Venterink, 2020; Sitters et al., 2014; van der Waal, Kool, et al., 2011). Dung and ca. 10 g fresh savanna soil was mixed through the quartz sand in each mesocosm prior to planting the seedlings. All treatments were carried out with two replicates as we analysed our data per plant functional type (i.e., N₂-fixing tree seedling vs. C₄-grass) and not per plant species. We therefore had six monocultures per plant functional type and 18 mixtures, with a total of 15 different plant species combinations. The total number of mesocosms was: 15 plant species combinations \times dung of 2 herbivore species \times 3 dung supply levels \times 2 replicates = 180 mesocosms (see Table S2 for an overview of the experimental design). The mesocosms were randomly placed in the greenhouse at the VUB in three blocks with every treatment occurring at least once within each block. The mesocosms received de-ionised water twice a week to prevent

water limitation (ca. 600 ml per week). Throughout the duration of the experiment the average temperature of the greenhouse was 26°C, relative humidity was 40% and the light period was from 7 am until 5 pm.

During the experiment, dead leaves and shoots were collected. After four months (8-14 May 2017), the plants were harvested. The number of nodules were counted on the roots of all tree seedlings (we are missing root nodule data from two individuals). Biomass of the harvested plants and the collected dead material was dried at 70°C for 48 hours and weighed.

Control experiments

Simultaneous to the tree-grass competition experiment above, we ran two control experiments. The first was a competition experiment adding mineral forms of N and P to enable the evaluation of the importance of N:P supply ratio on the competition between the tree seedlings (*V. drepanolobium* and *V. etbaica*) and grasses (*B. bladhii* and *P. mezanum*) as mineral N and P are readily available for plant uptake. Nutrient treatments consisted of two N:P supply ratios (1 and 9) at six levels of nutrient supply (0.03, 0.1, 0.3, 0.9, 2.7, 8.1 mg N mesocosm⁻¹) with one replicate each. Nutrients were supplied as salt solutions once a week with N supplied as NaNO₃ and P as NaH₂PO₄. Additionally, every week other essential nutrients were given in constant non-limiting amounts to all mesocosms. A total of 87.5 ml nutrient solution was applied to each mesocosm during the course of the experiment. As the plants' demands for nutrients increased with plant age, 2.5 ml of nutrient solution was given to each mesocosm in the first three weeks, 5 ml in the ten weeks in between, and 7.5 ml in the last four weeks. After four months, plants were harvested as described above. See Appendix S2 for a full description of the methods and Table S2 for an overview of the experimental design.

The second control experiment was established to examine the relationship between dung supply and nodule production in N₂-fixing tree seedlings. We grew one individual seedling of *Senegalia mellifera* or *V. tortilis* in 0.4 L mesocosms, fertilized with dung from giraffe or zebra supplied at nine levels (1, 2, 4, 8, 16, 32, 64, 128, 256 mg N mesocosm⁻¹) with one replicate each. Unfortunately, we did not have enough seedlings left of the *Vachellia* species used in the competition experiments described above, so we had to run the experiment with two new

species. After four months, root nodules were counted, and biomass was harvested. See Appendix S3 for a full description of the methods.

Calculations and statistical analyses

For both the tree-grass competition experiment and the control experiment with mineral nutrients, biomass of the dead leaves and shoots was added to the harvested plant biomass to get total plant biomass. We calculated the relative growth rate (RGR) per plant as $(\ln M_1 - \ln M_0) / (t_1 - t_0)$, whereby M is biomass at either the start of the experiment (M_0) or at harvest (M_1) and $t_1 - t_0$ the amount of days between start and harvest (Pérez-Harguindeguy et al., 2013). For the monocultures we averaged total plant biomass and RGR over the two plants per mesocosm to account for the presence of two vs. one plant of the same species in monocultures and mixtures, respectively. We did the same for the number of root nodules counted in the monocultures with tree seedlings.

We calculated relative competitive strength as ratios of RGR in a mixture mesocosm by that of the same species in a monoculture mesocosm. In a given dung or mineral nutrient treatment, a plant functional type was considered to be the superior competitor if its growth was significantly higher in mixture than in monoculture (competitive strength > 1) and/or cause the other plant functional type to grow significantly less in mixture than in monoculture (competitive strength < 1) (Minden & Olde Venterink, 2019). A similar ratio was calculated for the number of root nodules produced by tree seedlings in a mixture to monoculture to indicate if grass competition enhanced (nodule ratio > 1) or suppressed root nodule production (nodule ratio < 1).

For each plant functional type, we used linear mixed effect models (LMMs) to test the effects of dung type, dung amount and inter- vs. intraspecific competition (mixtures vs. monocultures) on total biomass, RGR and number of root nodules (only for N₂-fixing tree seedlings), with plant species nested within block as a random factor. We used three-way unbalanced ANOVAs due to differences in number of replicates between monocultures (N = 36) and mixtures (N = 108) and obtained type II Sums of Squares using the function 'Anova' in the *car* package in R, version 3.6.1 (R Core Team, 2019). The effect of plant functional type on total biomass and RGR was tested separately for monocultures and mixtures, as plants grown together

in a mixture mesocosm were not independent from each other. Plant species was therefore nested in mesocosm ID, which was nested in block, as a random factor, in the LMMs testing the effect of plant functional type for the mixtures. The effects of dung or mineral nutrient addition on the relative competitive strength of each plant functional type was tested using LMMs with dung type or mineral N:P ratio and N supply (either as dung or in mineral form) as fixed factors with interaction, and plant species nested within block as a random factor. These LMMs were run separately for the tree-grass competition experiment and the control experiment with mineral nutrients. Variables were transformed (log or square root) if necessary, to more closely meet assumptions of normality and homogeneity. We used the *nlme* package for mixed-effects modelling and made multiple comparisons using Tukey's tests in the *lsmeans* package.

We also tested if the relative competitive strength of each plant functional type for a certain nutrient treatment (dung or mineral) was significantly different from 1 using one-sample t-tests. We performed regression analyses to examine the relationship between N supply (in dung or mineral form) and relative competitive strength of each plant functional type for the different N:P ratios. In addition, we performed regression analyses to examine the relationship between the ratio of root nodules in mixture to monoculture and the relative competitive strength of tree seedlings. We also tested if the nodule ratio was significantly different from 1 for a certain dung treatment using one sample t-tests. For the control experiment on nodule production, regression analyses were performed to examine the relationship between dung N supply and number of root nodules.

Results

Tree-grass competition experiment

Growth of N₂-fixing tree seedlings was higher in monocultures compared to mixtures when giraffe dung (N:P = 6.4) was added, while C₄-grasses grew better in mixtures with this type of dung (Fig. 2; Table 1). When zebra dung (N:P = 3.5) was added, tree seedlings grew better in mixtures (Fig. 2a), while grasses showed no differences in growth between the competition treatments (Fig. 2b). The relative competitive strength of both plant functional types was thus affected by dung type as hypothesized: N₂-fixing tree seedlings were the superior competitor

when zebra dung was added, while grasses were the superior competitor when giraffe dung was added (relative competitive strength >1 ; Fig. 3; Table 2).

The relative competitive strength of N_2 -fixing tree seedlings responded as hypothesized to dung amount, while the grasses did not (Fig. 1c, d). Tree seedlings only gained competitive advantage over grasses when medium (10 mg N) or high (50 mg N) amounts of zebra dung were added, but not under a low amount of dung (2 mg; see asterisks in Fig. 3a). Hence, as expected, the relative competitive strength of tree seedlings peaked at a medium supply of zebra dung, after which performance decreased (Fig. 3c; 2nd order polynomial regression, $P < 0.001$, $R^2 = 0.25$). When giraffe dung was added, tree seedlings became more competitively disadvantaged with increasing amount (Fig. 3c; linear regression, $P = 0.002$, $R^2 = 0.15$). Grasses gained competitive advantage over tree seedlings at any amount of giraffe dung (see asterisks in Fig. 3b), but contrary to what we expected, their relative competitive strength also peaked at medium supply (Fig. 3d; 2nd order polynomial regression, $P = 0.026$, $R^2 = 0.10$), instead of increasing with amount. We did not find a significant impact of zebra dung on the competitive strength of grasses at any amount (Fig. 3b, d).

N_2 -fixing tree seedlings had a higher RGR than grasses and for both plant functional types RGR increased with dung amount (Table 1, Fig. S1). The range in RGRs under dung fertilization was comparable to the range under mineral fertilization (Fig. S1), indicating that the plants received sufficient nutrients throughout the experiment from our single dung addition event. For tree seedlings, RGR showed a stronger increase with dung N supply when grown in monocultures under giraffe dung or in mixtures under zebra dung (Fig. S1a; significant three-way interaction in Table 1). For grasses, the increase in RGR with N supply was similar across dung types and competition treatments (Fig. S1b), although not significant for grasses in monocultures under giraffe dung. This indicates that changes in the relative competitive strength of tree seedlings and grasses within the different dung treatments were driven by changes in the RGR of tree seedlings and not grasses; e.g., higher relative competitive strength of tree seedlings under zebra dung due to higher RGR in mixtures, lower competitive strength of tree seedlings under giraffe dung due to higher RGR in monocultures.

N_2 -fixing tree seedlings produced more root nodules under zebra dung compared to giraffe dung (Table 1, Fig. S2). The number of nodules increased with dung amount, but this was

merely a result of larger plants, as there was no relationship between dung amount and nodule number per unit of total biomass (Table 1 and also see Fig. S3 for positive correlation between number of nodules and total tree seedling biomass). We found a positive relationship between the ratio of root nodules in mixture to monoculture and the relative competitive strength of tree seedlings, especially under medium and high amounts of zebra dung (Fig. 4). Under these dung treatments, grass competition likely induced N-limitation, which in turn enhanced nodule production (nodule ratios > 1 ; $t = 2.8$, $P = 0.01$ for medium amount and $t = 4.0$, $P < 0.001$ for high amount), increasing the relative competitive strength of tree seedlings (Fig. 4).

Control experiments

In the control experiment with mineral nutrients, N_2 -fixing tree seedlings also had a competitive advantage when receiving mineral nutrients with a low N:P ratio of 1, especially when higher amounts of N were added (relative competitive strength > 1 from $0.9 \text{ mg N mesocosm}^{-1}$; Appendix S2). C_4 -grasses had a competitive advantage over tree seedlings at a high N:P ratio of 9.

In the control experiment on nodule production, the number of root nodules per N_2 -fixing tree seedling peaked at intermediate levels of giraffe and zebra dung (Appendix S3).

Discussion

Our hypothesized concept about the role of herbivore dung on competitive interactions between N_2 -fixing trees and grasses in savanna ecosystems (Fig. 1) was supported by the results of our competition experiment, albeit more strongly for the relatively novel aspect of dung stoichiometry than for the more classical aspect of dung amount. As we hypothesized, N_2 -fixing tree seedlings had a competitive advantage over grasses when receiving dung with a low N:P ratio (zebra dung), while grasses had a competitive advantage when receiving dung with a high N:P ratio (giraffe dung) (Figs. 2, 3). Our results provide the first experimental evidence that the balance between N_2 -fixing trees and grasses – by definition a crucial characteristic of savanna ecosystems – is impacted by differences in dung N:P stoichiometry of the herbivores that feed on these two functional groups of savanna plants, at least during early stages of plant development and competition.

The results of our competition experiment clearly demonstrate that dung type (from giraffe or zebra) had opposite effects on the outcome of competition between tree seedlings and grasses in savanna. In our hypothesized concept we ascribe this effect to the differences in dung N:P ratio between browsers and grazers (Sitters & Olde Venterink 2018; Fig. 1), and obviously the difference between N:P = 6.4 (giraffe) and N:P = 3.5 (zebra) is already large enough to induce an effect on tree-grass interactions. That we observed similar patterns in our control experiment with mineral nutrients – where N₂-fixing tree seedlings or grasses had a competitive advantage at a N:P ratio of 1 or 9, respectively (Appendix S2) – strongly suggests that competition is indeed driven by N:P supply ratio. We chose dung from giraffe and zebra for our experiment because these species represent ‘typical’ browsing and grazing herbivores in African savanna. However, even though the N:P ratio of the zebra dung used, was similar to the mean N:P ratio of grazer dung collected in the same Kenyan savanna (3.5 ± 0.2 vs. 3.7 ± 0.5), the giraffe dung had a higher N:P ratio than the mean N:P ratio of browser dung (6.4 ± 0.4 vs. 5.0 ± 0.4) (Fig. 1b). The difference in N:P ratio between browser and grazer dung in this Kenyan savanna was relatively small compared to other savannas (8.9 vs. 4.8 in Kruger, South Africa; Grant et al. 1995; 8.3 vs. 4.7 in Saadani, Tanzania; Sitters et al. 2014), hence our competitive effect can also be expected in other savannas, but one should be careful with generalizations based on individual herbivore species since dung N:P ratio varies within the same functional group of herbivores (Sitters & Olde Venterink, 2021a).

The outcome of the competition between the N₂-fixing tree seedlings and grasses was driven by changes in RGR of the tree seedlings and not the grasses (Fig. S1a). In turn, these changes in RGR and hence relative competitive strength of the tree seedlings were related to their nodulation rates (Fig. 4). All three tree species in our experiment were actively nodulating and we used the number of nodules as a proxy for the rate of N₂-fixation (McNeill & Unkovich, 2007). Hence, in line with our hypothesized concept (Fig. 1), we argue that the tree seedlings gained competitive advantage under low N:P dung due to their increased ability to fix N₂. Indeed, tree seedlings produced on average more nodules under low N:P zebra dung compared to high N:P giraffe dung (Fig. S2). Tree seedlings outcompeted grasses when supplied with medium (10 mg) or high (50 mg) amounts of zebra dung (Fig. 3a), which was related to an increase in nodule production induced by grass competition (ratio root nodules mixture to mono > 1 at medium and

high amounts of zebra dung; $t = 4.6, P < 0.001$; Fig. 4). Under a low amount of zebra dung (2 mg N), the production of root nodules was likely still limited by P, as plants had nearly 40% less nodules compared to the medium and high zebra dung supply, which prevented tree seedlings from gaining a competitive edge over grasses (Fig. 3a). Grasses likely experienced N-limitation at low zebra dung supply and were therefore also not able to gain a competitive advantage (Fig. 3b). Our results are supported by experimental studies of Cramer and colleagues, who showed that grass competition induced root nodulation in several species of African *Acacia* thereby enhancing N₂-fixation rates (Cramer, Chimphango, van Cauter, Waldram, & Bond, 2007), and that the competitive suppression of N₂-fixing tree seedlings by a grass sward was reduced under increased P availability (Cramer, van Cauter, & Bond, 2010).

We expected nodule production of our tree seedlings – and consequently their relative competitive strength – to peak at some intermediate supply of dung (Fig. 1b). At this dung supply, nodule production would no longer be constrained by low P, but not yet be inhibited by high N (Barron, Purves, & Hedin, 2010; Batterman et al., 2013; Finzi & Rodgers, 2009; Pons, Perreijn, van Kessel, & Werger, 2007; Vitousek et al., 2002). Indeed, our control experiment showed that the overall nodulation rate peaked at intermediate dung supply (Appendix S3). From the control experiment we can deduce that under the highest dung supply level in our main experiment (50 mg, which is up to 1.7 on the log scale of Fig. 1 in Appendix S3), nodule production was likely still constrained by low P and not yet reduced by too much N. We therefore argue that even under our highest supply level of low N:P zebra dung, tree seedlings had not yet lost their competitive edge to grasses as predicted in Fig. 1. We conclude that the element of N₂-fixation in our hypothesized concept was supported by our experimental results, illustrating that the availabilities of both N and P in herbivore dung control nodule production and N₂-fixation rates of the legumes (cf. Batterman et al., 2013; Houlton, Wang, Vitousek, & Field, 2008; Vitousek, Porder, Houlton, & Chadwick, 2010).

Nodule production of the N₂-fixing tree seedlings was suppressed by grass competition under high N:P giraffe dung (ratio root nodules mixture to mono < 1 at medium and high amounts of giraffe dung; $t = -4.6, P < 0.001$; Fig. 4), which in turn suppressed tree seedling growth. As predicted, C₄-grasses outcompeted the tree seedlings under these more P-limited conditions (Fig. 3). However, the competitive advantage of grasses receiving giraffe dung peaked

at medium supply (Fig. 3d), which contrasted with our prediction of an increase with dung amount (Fig. 1d). This pattern was also observed in the control experiment with mineral nutrients under high N:P ratio (Appendix S2), which may point out that the competitive difference between the grasses and tree seedlings was due to a higher N use efficiency of the grasses, rather than to a higher soil N acquisition capacity. In the latter case we would have expected a linear increase as predicted, which was observed for the tree seedlings competing for soil P (Appendix S2), although more data about soil nutrient acquisition and nutrient efficiency traits are needed to further explore this mechanistic explanation.

We evaluated whether other differences than N:P ratio between zebra and giraffe dung might have been responsible for the observed competitive outcome between grasses and tree seedlings in our experiment but did not find evidence for that. Firstly, theoretically the release rates of N and P might be constrained by how much of the N and/or P is locked up in secondary metabolites (e.g., tannins, lignin), which are known to slow down decomposition rates (Chomel et al., 2016; Hattenschwiler & Vitousek, 2000), but we did not find differences in phenol or tannin concentrations between our two dung types (Fig. S4). Secondly, fractions of directly available N and P might be different among dung types; in our case, zebra dung did have higher extractable nitrate, ammonium and phosphate contents than giraffe dung, but the difference in extractable N:P ratio between dung types was similar to the difference in total N:P ratio (i.e., N:P ratio of giraffe dung nearly twice that of zebra dung; Table S3). Thirdly, differences in structural and moisture characteristics of the dung as occurring under field conditions, did not play a role in our experiment where we applied dried dung ground into powder.

This tree-grass competition experiment focusing on the use of organic fertilizers in the form of herbivore dung is the first of its kind, and a good start to gain insights into the role of herbivore dung on ecosystem functioning. By replicating our plant functional types (i.e., N₂-fixing trees and C₄ grasses) with three different species we were able to generalize our results to a certain extent. We focused on N₂-fixing tree species as they are especially abundant in African savanna (ca. 40%; Pellegrini et al., 2016). In the Kenyan savanna where we collected our plant seeds and herbivore dung, the species *Vachellia drepanolobium* can reach overstorey cover of >97% (Young et al. 1995). However, we acknowledge that non-fixing tree species are another plant functional type that occur in African savanna, which were not included in our experiment.

These tree seedlings will likely respond differently to grass competition under dung fertilization as they for example will be more limited by N than P compared to N₂-fixing tree seedlings (Varma et al., 2018).

An important next step is to test tree-grass competition in the field under influence of different types of herbivore dung. Under field conditions, dung decomposition rates depend on decomposing organisms, such as dung beetles and termites, of which the first are for example more attracted to zebra than giraffe dung (Sitters et al., 2014). Also, in our experiment the only nutrients available to the plants were from herbivore dung, but there will be a plant-available soil nutrient pool in the field. Based on several studies from African savanna, the plant-available soil N and P pools range from 0.2-1.4 g m⁻² and 0.2-0.7 g m⁻², respectively, depending on soil type, cover of N₂-fixing tree species and level of herbivore dung deposition (Augustine et al., 2003; Augustine & McNaughton, 2006; Cech, Olde Venterink, & Edwards, 2010; Sitters, Kimuyu, et al., 2020; van der Waal, Kool, et al., 2011). The plant-available soil N pool is of similar magnitude to the dung N pool (0.04-1.2 g m⁻²) in these savannas and they are positively correlated (Pearson's $r = 0.84$, $P = 0.017$; see Table S4 for values). Indeed, addition of dung will increase plant-available nutrient pools in the soil, stimulating plant growth and quality, and changing plant species dominance (Blackmore, Mentis, & Scholes, 1990; McNaughton, Banyikwa, & McNaughton, 1997; Porensky & Veblen, 2015; van der Waal, Kool, et al., 2011). Consequently, we argue that competitive interactions between plants in the field are likely frequently influenced by dung nutrient supply levels as in our experiment, and that plants will respond more strongly to dung fertilization in areas with low soil nutrient availabilities. Also, water availability was never limiting in our experiment, but is likely an important factor for tree-grass competition under field conditions (Kraaij & Ward, 2006; Morrison, Holdo, Rugemalila, Nzunda, & Anderson, 2019; Sankaran & Augustine, 2004). Furthermore, our experiment only looked at the competitive outcomes between trees and grasses in the seedling stage and these might be different for adult plants, for example because of separation in rooting depth (Scholes & Archer, 1997). However, the dynamics of savanna systems are strongly related to tree seedling establishment and growth, as they are the most vulnerable in this life-history stage (Bond, 2008; van der Waal, de Kroon, et al., 2011). In the field, tree seedlings often need to establish and grow in grass swards where they likely suffer from more severe competition (van der Waal et al., 2009), although they also

get dispersed and established in dung piles of herbivores, which might benefit them (Reid & Ellis, 1995; Tjelele, Ward, & Dziba, 2015).

Tree-grass coexistence in savannas has been attributed to competition for water, nutrients, and disturbances including fire and mammalian herbivores (Sankaran et al., 2005). In our experiment, we excluded the roles of water, fire and herbivore consumption, creating the chance to solely focus on the role of herbivore dung. The results support our hypothesized concept that the stoichiometric differences in dung of browsing and grazing herbivores helps maintain the balance of trees and grasses in African savanna. We added dung amounts to our mesocosms that were comparable to dung pools at several locations in African savanna (Table S4), which suggests that our experimental results are applicable to field conditions. However, in the field, the spatial redistribution of nutrients in the form of dung is patchy, suggesting that our observed competitive outcomes between tree seedlings and grasses might mainly occur at a smaller scale within these nutrient-enriched patches (Augustine & Frank, 2001; Hobbs, 1996). To maintain and regulate the tree-grass balance in savanna ecosystems, the balance between grazer and browser metabolic biomass is essential due to a multitude of feedback loops from these large herbivores to plants (du Toit & Cumming, 1999). So far, the focus has been on the impact of these two groups of herbivores on the tree-grass balance directly through their forage activities and indirectly through changes in fire regimes (Archibald et al., 2005; Goheen et al., 2010; Kimuyu et al., 2014; Roques et al., 2001; Staver & Bond, 2014; van Langevelde et al., 2003). The study presented here identifies another potentially important mechanism by which these herbivores can stimulate ecosystem stability, namely through the N:P ratio of their dung. As dung is dropped continuously at different locations, a shifting mosaic of N:P availability is created, facilitating tree-grass coexistence.

Grasses and leguminous plants – be it trees or forbs – are generally important components of plant communities in grazing ecosystems worldwide. Our case study in African savanna clearly illustrates the impact that herbivore dung stoichiometry has on competition between these functional groups of plants, but we have indications that this concept is not unique for this ecosystem, as we found similar effects using European species (Valdés-Correcher et al., 2019). Hence, the concept appears to be more widely applicable, but there is need for further testing in other grazing ecosystems, particularly under field conditions. Our concept,

which shows that connections between N₂-fixing trees and grasses, as well as the herbivores that feed on them, help maintain the tree-grass balance, illustrates that the influence of these ecosystem components on ecosystem functioning is complex, and can easily be disturbed when herbivore species get lost due to anthropogenic interferences (Sitters, Wubs, et al., 2020; Sitters, Kimuyu, et al., 2020).

Acknowledgements

This research was carried out under Government of Kenya research clearance permit no. NACOSTI/P/15/0830/4886. We thank DM Kimuyu, TP Young and B Kimiti for their assistance in the field, and J Hene, AA Along and C Moyersoen for laboratory work. We also acknowledge the staff at Mpala Research Centre and the KLEE team for their logistical support. JS was funded by grants from the Research Foundation Flanders (FWO), grants 12N2615N and 12N2618N, and the Leopold III Fonds voor Natuuronderzoek en Natuurbehoud.

Authors' contributions

JS and HOV designed and conducted the experiments. JS collected plant seeds, soil and herbivore dung. JS analysed the data. JS and HOV wrote the manuscript.

Data availability

Data available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.h9w0vt4h4> (Sitters & Olde Venterink, 2021b).

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Figure legends

Fig 1. (a) Conceptual framework of African savanna where the stoichiometric differences in dung from browsing and grazing herbivores helps maintain the balance between N₂-fixing trees and grasses. (b) Browsers produce high N:P dung because they consume tree leaves with high N:P ratios, while grazers produce dung with lower N:P ratios as they consume grasses with lower N:P ratios. Panels (a) and (b) were adapted from Fig. 1 in Sitters and Olde Venterink (2018). Panels (c) and (d) show hypothesized effects of dung N:P ratio and amount on the competitive strength of N₂-fixing tree seedlings and C₄-grasses, respectively. Values >1 indicate a superior competitor as then a plant functional type produced significantly more biomass in mixture than in monoculture and/or caused the other plant functional type to produce significantly less biomass in mixture than monoculture.

Fig. 2. Effect of dung from giraffe (orange boxes) and zebra (blue boxes) on RGR of (a) N₂-fixing tree seedlings and (b) C₄-grasses grown in monocultures (two individuals per mesocosm; light grey boxplots) or in mixtures (one individual of each plant functional type per mesocosm; dark grey boxplots). RGR values in the monocultures were averaged for comparability. Boxplots include the median and their whiskers the minimum and maximum values (N = 18 for monocultures and N = 54 for mixtures). Asterisks indicate significant differences between monocultures and mixtures for a certain dung type per plant functional type with ** $P < 0.01$ and *** $P < 0.001$. Results of the three-way unbalanced ANOVAs are shown in Table 1.

Fig. 3. Relative competitive strength of (a, c) N₂-fixing tree seedlings and (b, d) C₄-grasses for the three amounts of N supplied (2, 10 or 50 mg N mesocosm⁻¹) by dung from giraffe (orange boxplots, points and lines) and zebra (blue boxplots, points and lines). Relative competitive strength was calculated as ratios of RGR in a mixture mesocosm by that of the same species in a monoculture mesocosm. A plant functional type was considered to be the superior competitor if its competitive strength > 1 and/or if the other plant functional type had competitive strength < 1. (a, b) Boxplots include the median and their whiskers the minimum and maximum values (N = 18). Asterisks indicate if competitive strength was different from 1. (c, d) Linear or 2nd order polynomial regression lines were drawn where significant to show the relationship between N

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supply (log-transformed) and relative competitive strength (see text for significance levels and R^2 values).

Fig. 4. Relative competitive strength of N_2 -fixing tree seedlings plotted against the ratio of root nodules in mixture to monoculture for the three amounts of N supplied by the different dung types (orange points and lines: giraffe dung; blue points and lines: zebra dung; $N = 18$). Linear regression lines were drawn where significant with: $P < 0.001$, $R^2 = 0.63$ for giraffe dung at 10 mg N; $P = 0.016$, $R^2 = 0.27$ for zebra dung at 10 mg N; $P = 0.04$, $R^2 = 0.20$ for zebra dung at 50 mg N.

Table 1. ANOVA results (*F*-values and significance levels) for the effects of dung type, dung amount (N supply), competition (inter- vs. intraspecific) and plant functional type on total biomass, relative growth rate (RGR) and number of root nodules of N₂-fixing tree seedlings and C₄-grasses.

	Total biomass (g)	RGR (g ⁻¹ g ⁻¹ day ⁻¹)	# Nodules	# Nodules g biomass ⁻¹
<i>N₂-fixing tree seedlings</i>				
Dung type (DT)	29.2***	13.8***	5.2*	3.5
Dung amount (DA)	91.0***	63.9***	21.2***	1.7
Competition (Co)	0.36	0.2	0.3	0.004
DT x DA	9.4**	6.5*	5.9	2.0
DT x Co	40.3***	25.7***	7.7**	2.6
DA x Co	2.2	0.1	0.3	1.9
DT x DA x Co	20.2***	14.5***	7.2*	0.5
<i>C₄-grasses</i>				
Dung type (DT)	7.2**	7.5**		
Dung amount (DA)	76.5***	66.9***		
Competition (Co)	0.6	8.1**		
DT x DA	10.4**	7.7*		
DT x Co	16.5***	15.5***		
DA x Co	9.0*	4.5		
DT x DA x Co	9.5**	2.9		
<i>Monocultures</i>				
Dung type (DT)	0.1	0.5		
Dung amount (DA)	14.7***	26.8***		
Functional Type (FT)	90.1***	21.5***		
DT x DA	1.3	2.2		
DT x FT	7.3**	14.2***		

DA x FT	0.8	3.0
DT x DA x FT	2.8	2.4

Mixtures

Dung type (DT)	2.9	0.3
Dung amount (DA)	29.2***	26.8***
Functional Type (FT)	878.3***	100.4***
DT x DA	0.2	0.2
DT x FT	32.3***	24.3***
DA x FT	0.08	0.2
DT x DA x FT	7.1**	5.5**

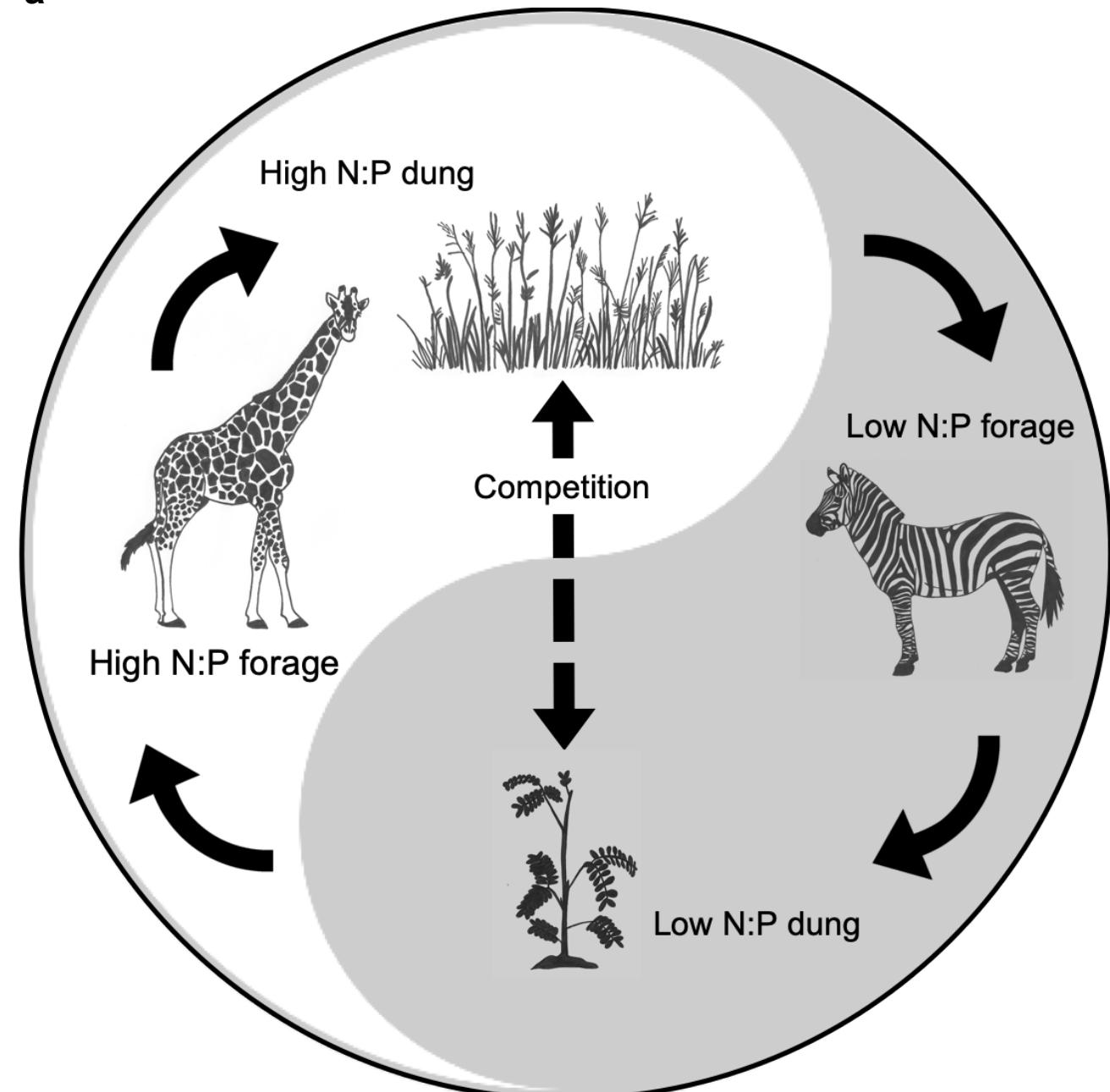
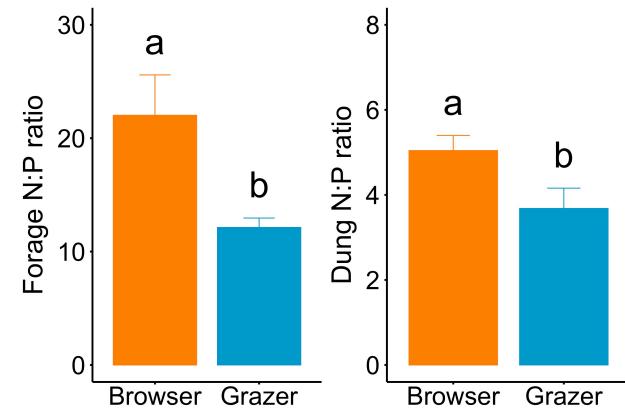
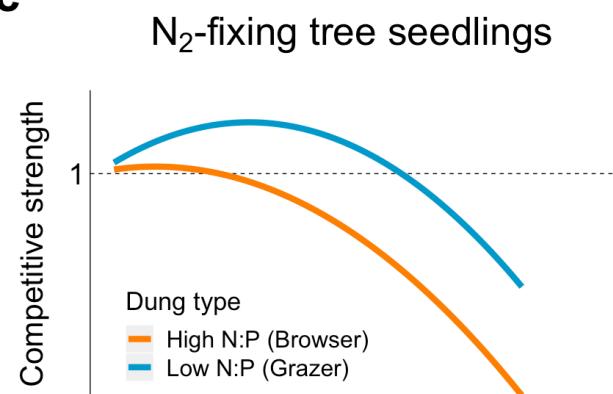
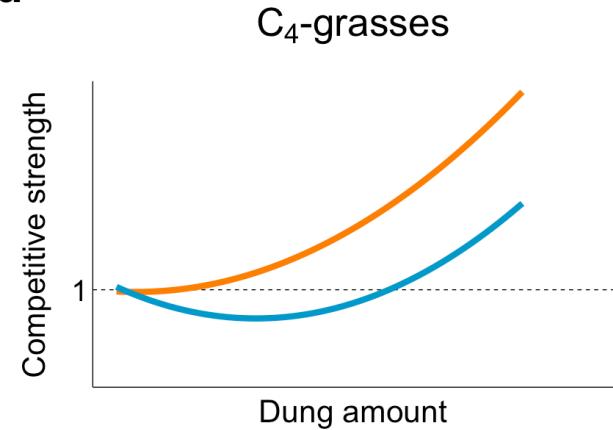
n = 6 for all plant traits. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

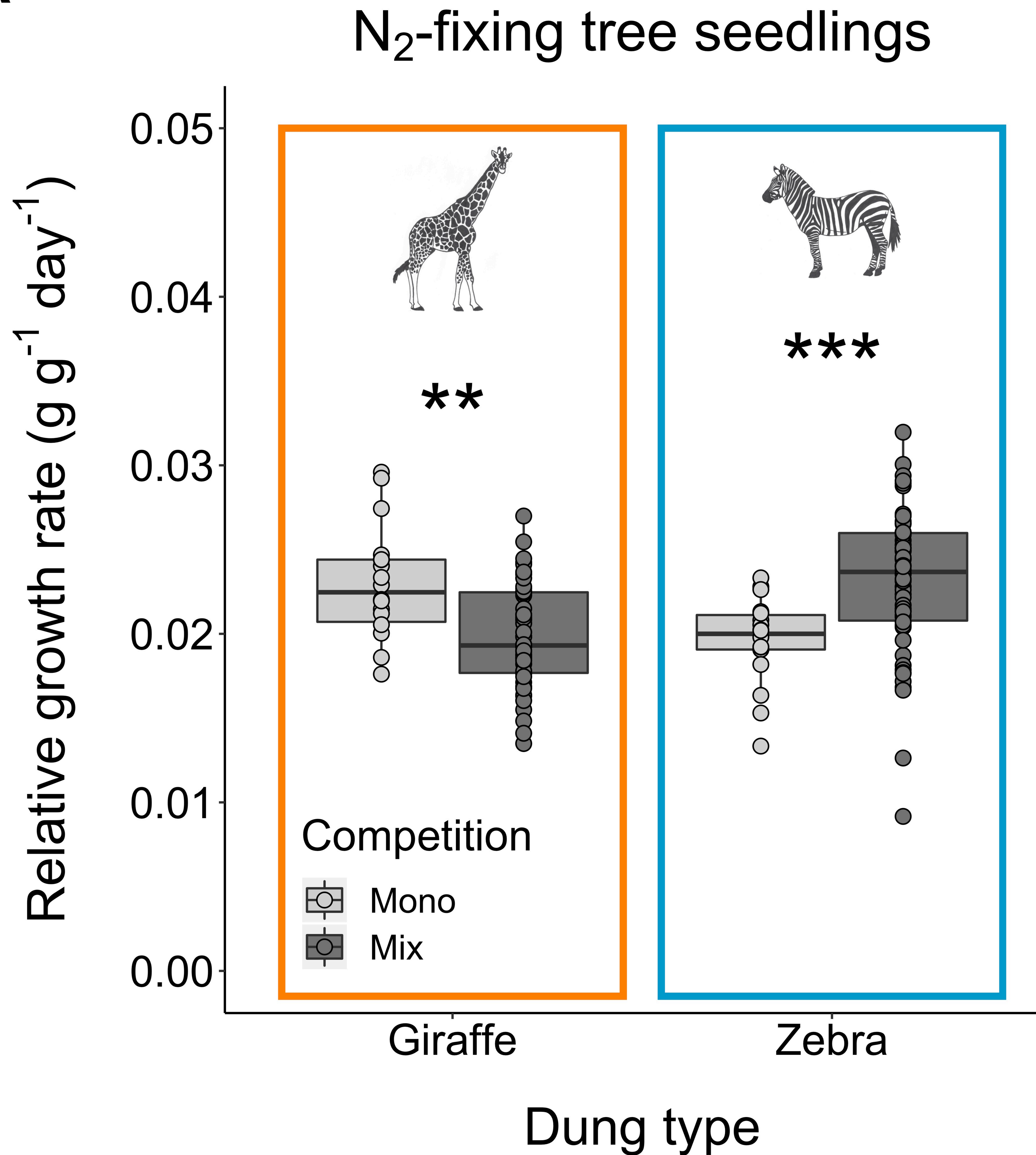
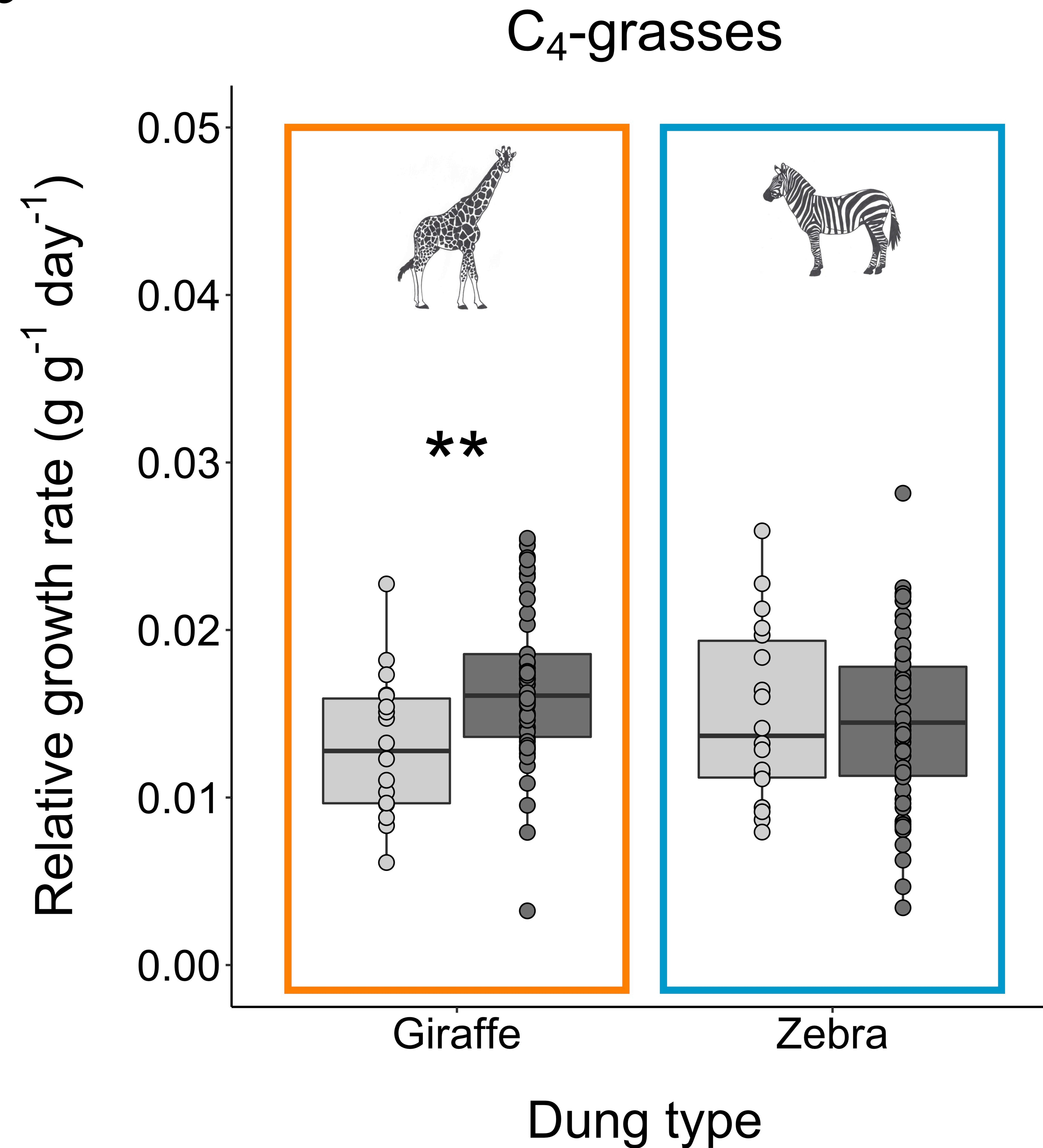
Table 2. ANOVA results (*F*-values and significance levels) for the effects of dung type (giraffe or zebra), dung amount (2, 10 or 50 mg N mesocosm $^{-1}$) and their interaction on the relative competitive strength of N₂-fixing tree seedlings and C₄-grasses.

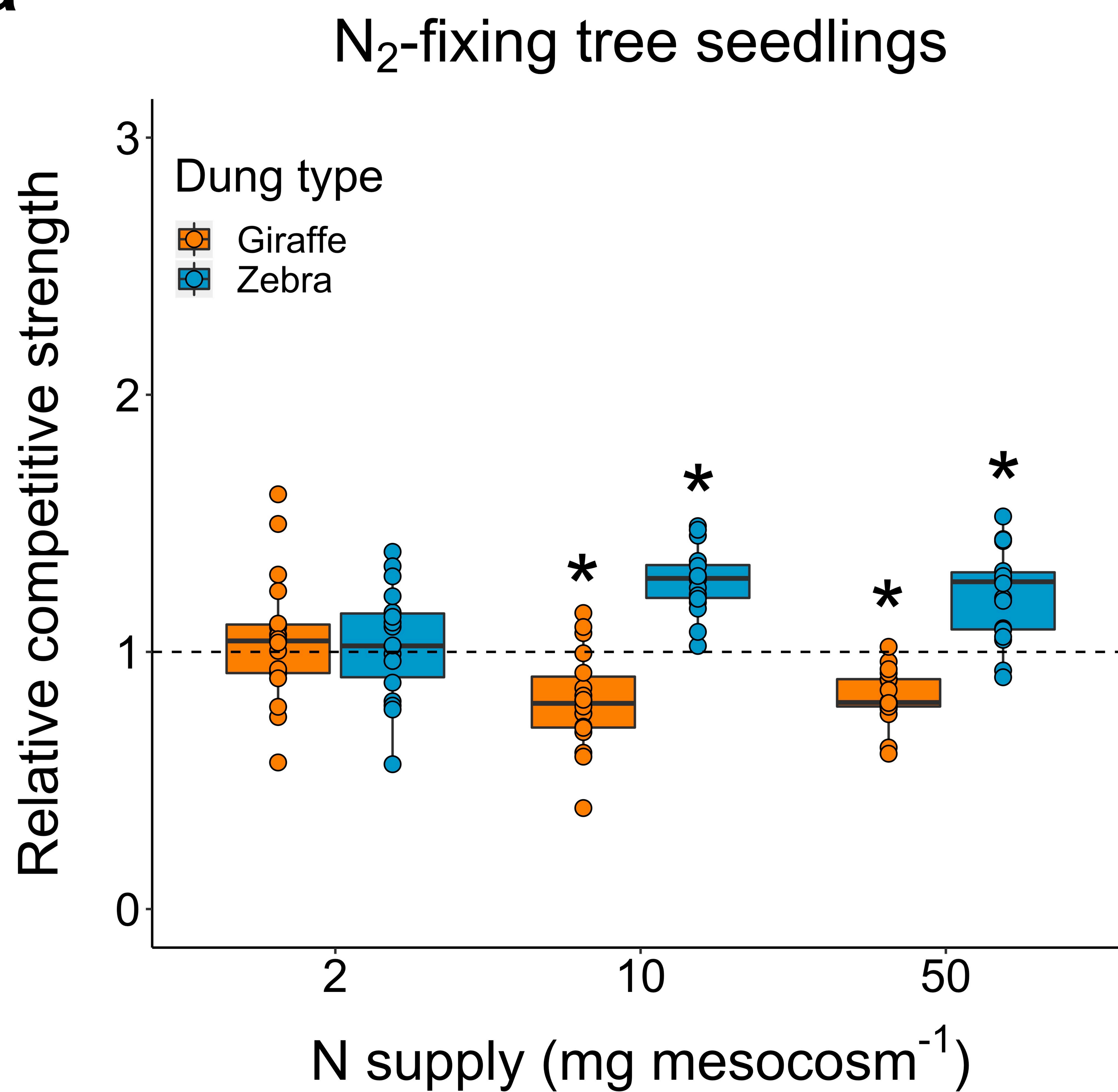
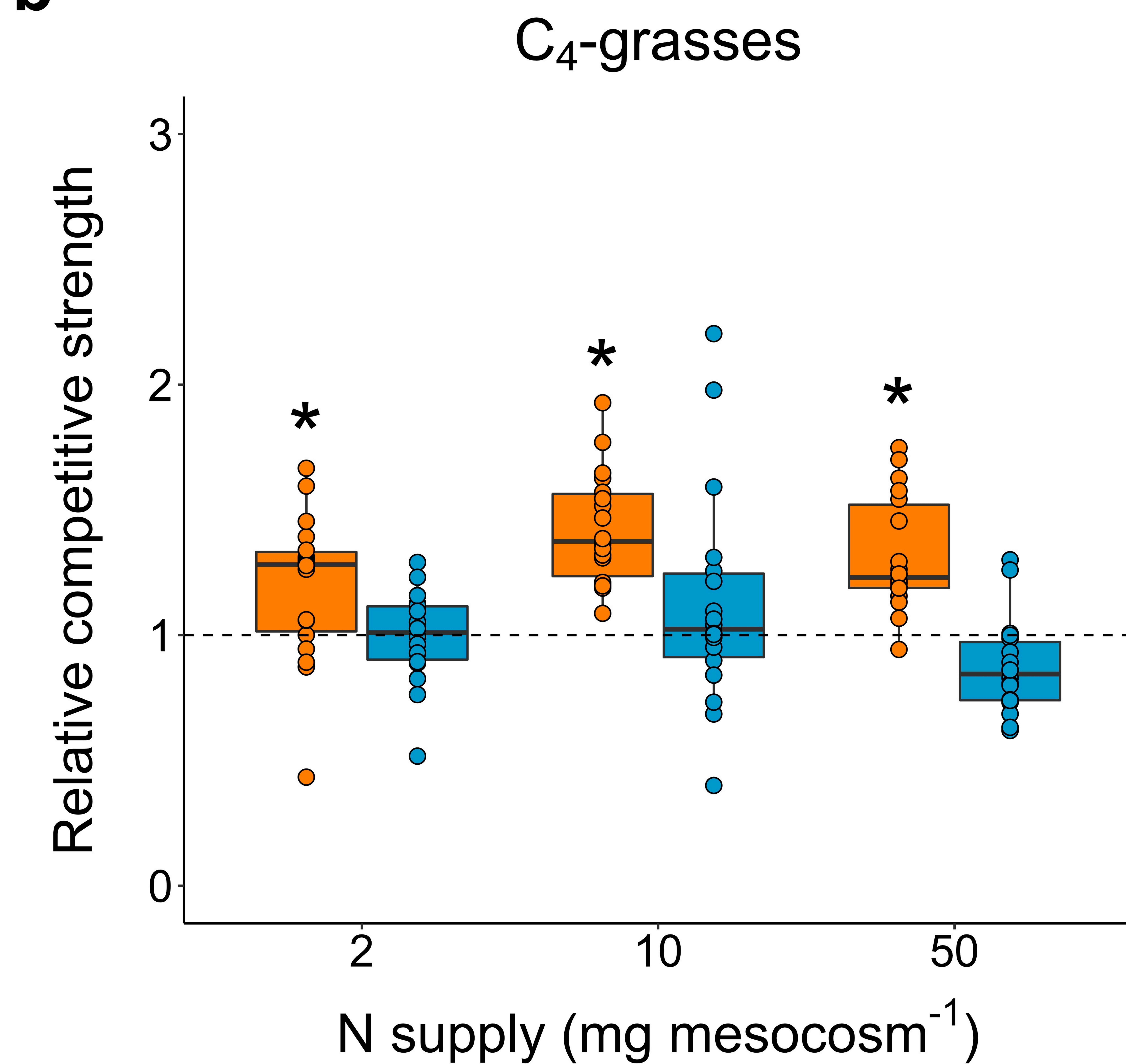
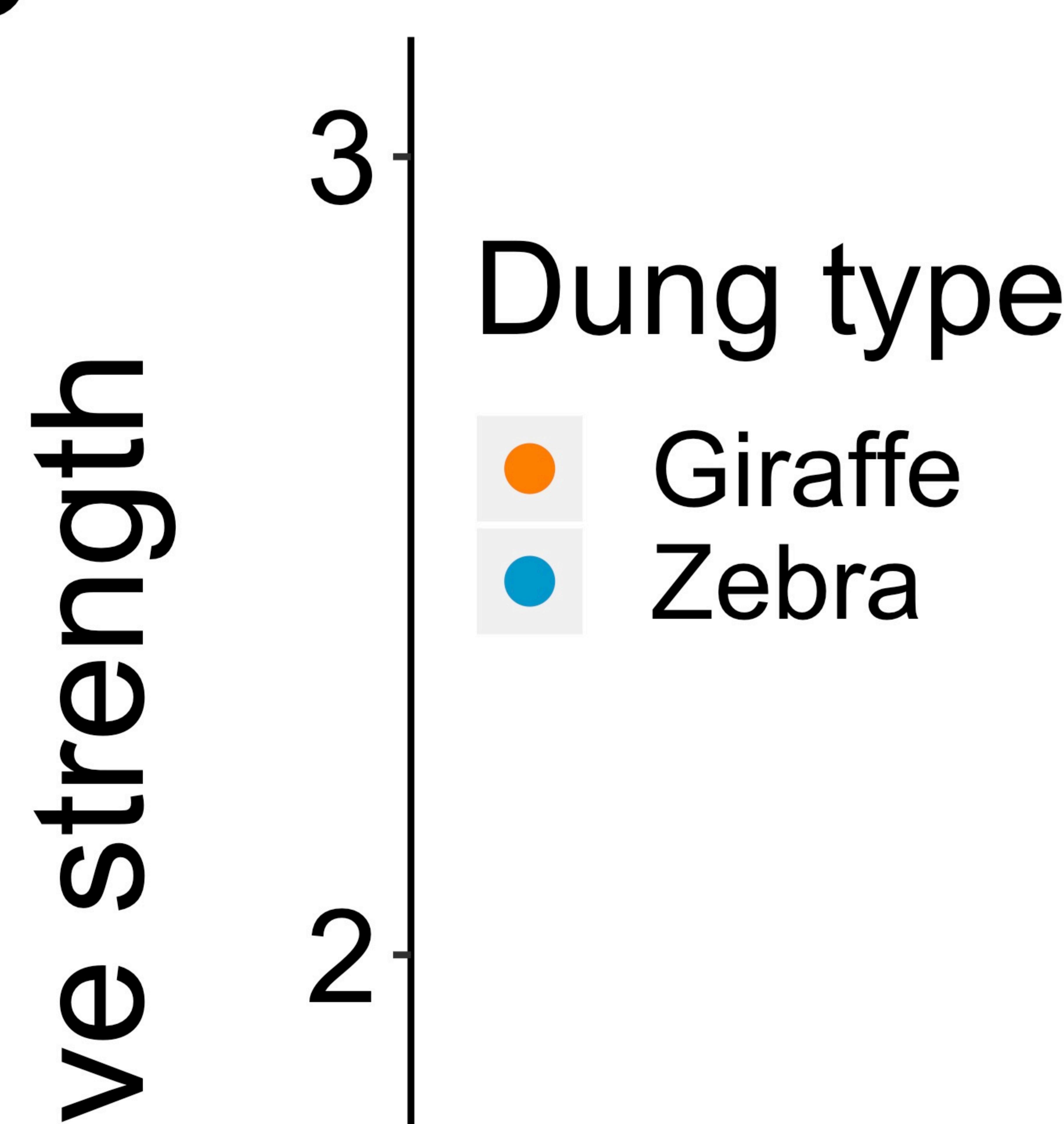
	Dung type (DT)	Dung amount (DA)	DT x DA
N ₂ -fixing tree seedlings	63.4***	0.06	18.1***
C ₄ -grasses	37.2***	4.7*	2.2

n = 18 for each dung type and amount per plant functional type.

*** $P < 0.001$, ** $P < 0.01$.

a**b****c****d**

a**b**

a**b****c****d**