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Interactive comment on “Altered response to nitrogen supply of mixed grassland communities in a future climate: a controlled environment microcosm study” by J. Van den Berge et al.

Anonymous Referee #1

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The manuscript reports on the response of artificially assembled temperate grassland species, (6) to five levels of nitrogen supply (0 to 150 kgNha⁻¹), grown under two climate scenarios. Compared scenarios were “current” temperature (T) and CO₂ (380ppm) and “future climate” with T+3°C and 620ppm. Water supply was the same as under current climate! Main findings, grassland communities (6 species) do not significantly respond to N addition in a future climate. I read this manuscript with interest and was waiting for some trade-offs. The manuscript has the potential to be an interesting paper. However, main discussion parts are speculative as explicative variables (i.e. supplied N did not contribute plant biomass N because atmospheric N₂-fixation declined and microbial biomass increased) were not measured in the present contri-

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bution (neither N₂O nor leaching). Accordingly, authors may go a bit further in giving estimates of those “missing variables” (see general comments). The manuscript needs revision I, thus, suggest to accept the MS, providing major revisions/resubmission.

General comments -Right at the beginning Authors talk a lot about climate change. This wording, however, is a summary of various circumstance. Authors should better define what they are looking at. Climate change (experiments) can be: extreme events, drought, temperature CO₂ ect. Accordingly, abstract misses that part, also some of the introduction parts are a bit vague . -I believe, that author could have gone further in there statistical analyses: I do not think the comparison of slopes by ANCOVA (Fig 3 and 5) is a good approach. Some of the regression line had P<0.05 so I wonder if the slope was significantly different between treatment and within the treatment (P3587L14-17). According to ANCOVA (3587L14) no climate × nitrogen interaction was found. However, if authors like to test this, they should rather apply a comparison of regression lines (ex. Statgraph Inc) which applies an ANOVA to slopes and intercepts. Nevertheless, authors can not describe no difference and say 4 lines later L 17:” This suggests that small differences between the slopes in the two climatesmay have contributed to the significantly weaker response of total community biomass to N supply”. (See also next paragraph) NOT all species seems to be affected by CO₂ and T (N zero treatment). Authors do not mention this and should consider this at the beginning of RESULTS section. -A long the same lines, rather then showing Table 1 and 2 with ANCOVA of all data. I would have preferred to see letters in Figs (1, 2, 4) showing the differences between scenarios for each variable (shoot, root, N ect) by using a simple ANOVA comparing current vs future climate for each N-level. This seems more illustrative than a summary table with DF, F and p-values, which does not show in which DIRECTION! and WHERE! scenarios played a role. -I also miss a paragraph on species strategy to cope with “future climate”. Some species seem unaffected by CO₂ and T, which is interesting (ex Lotus, Poa). In this conteaxt I wonder if authors should not apply the term functional groups rather than plant communities. -I did not understand what authors wanted to show with Fig 5 and 6. Fig 5 shows that

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SEN stays unchanged independent of N supply, which indicated that applied N is not in the soil anymore. Some N may have left the system (Leaching) ? In this context I wonder if it would be helpful to have a table with tot and species biomass (g), or at least N% instead of gN m⁻². Future climate seems to have an impact on biomass and N at 1 gN m⁻². How do authors explain that? -What were min/max T in the chamber. Did T extremes explain that future climate treatment did not uptake more N. As SWC, and VPD were similar, drought can not be responsible. -Authors should probably show a Fig with T, SWC of the study period. - At several locations reader gets impression that authors mention their personal thoughts/critics/justification (3582L9-12; 3583 L8-10). However, this gives the manuscript an strange nuance. -Objectives are difficult to read and needs rewording: To analyse the role N supply future climate, grassland communities were fertilised with different levels of N and subjected to either current climate or elevated CO₂ and warming (future climate) in climate-controlled chambers. The grassland community was created artificially by assembling species of different functional groups (grass, dicots, roset, leguminouse), such that responses arose of species arose from both species-specific sensitivities and interspecific interactions. The following hypotheses were tested: (1) climate change alters biomass responses of grassland communities to N supply; (2) this alteration is mediated by shifts in species composition. THOSE shifts were not discussed -M&M: Experimental set-up is sometimes confusing do to repeats and order (see specific comments) -N was applied in 4 splits. WHEN? -Discussion, comprises two big parts: under high CO₂ and T, Nfert i) replaces biological N fixation and ii) Nfert was fixed by microbes. In both cases Nbalance in future climate is the same than in the control treatment. This may be a reason, however, N was applied in 4 splits (3585L12) so I wonder if this may be the case 4 times!! Concerning i) FertN replace BioFert N, I am not convinced. If this is the case authors should estimate the N-fixation of leguminous during experiment, to see if values are comparable. ii) if microbial assimilation is that important, I wonder why it was not considered in the experimental design (or at least analyses of soil N ect). I would also include iii) N being emitted as NO₂/NO_x and iv) leaching. This

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discussion part is missing. Again authors should estimate N emitted during experiment using a model with soil T and water filled pore space (ex. Flechard et al . 2007) -Conclusion. ...” grassland communities do not significantly respond to N addition in a future climate” this is true, but, 6 species are probably not representative for a real permanent/temporal grassland community. Moreover I miss a sentence on effect of CO₂+T only (i.e. 0 N treatment.)

Specific Comment -3580 L7 “This altered response wasnot the result of a changing response from a single species, but all species seemed to contribute to it.” I am not convinced on that seeing Fig 1and 2. -3580 L15 .In regions where water is not limited, nitrogen (N) is one of the key limiting elements (Vitousek et al., 1997). FOR WHAT? -L17 arises whether the impact of climate change will.. CLIMATE CHANGE is large, WHAT WILL CHANGE? L23 only to themselves but also to other species via transfer.. WHAT transfer? -3581L1 ...Such effects of climate change, that .. -L4 ... soil mineral N pool; such as changes in mineralization of soil organic matter (SOM) and immobilization of N in SOM, in addition to uptake of -L7 ...driven by microbial activity and can be affected directly or indirectly by climate change HOW and in which direction? -L28 warming and N supply have been less investigated, and combined CO₂ – temperature – N studies are even rarer. CITATION -3582L17-25 needs rewording!!: The Experiment was carried out in 6 sunlit, climate controlled chambers, which are part of a larger experimental platform. The platform is located at University of Antwerp, Belgium (51_090 N, 04_240 E). Mean annual precipitation is 776mm (evenly distributed throughout the year) and mean annual air temperature is 10.8 _C. Chambers (150x150cm) were orientated south and distances between chambers were maximized to avoid mutual shading. Chamber height at north and south side was 150 and 120 cm. Top of the chambers was a colorless polycarbonate plate (4mm thick), and sides were made of polyethylene film (200mm thick), both UV transparent. Three of the six chambers were run under current climate with current air temperature (T_{air}) and CO₂ concentration and remaining three chambers were exposed to a future climate scenario with 3 ... -3583 L8-10 delete -L10-14 The CO₂ concentration in chambers was measured and

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regulated by a CO₂ control group connected to CO₂ analyzer (WMA-4, PPSystems, Hitchin, UK). In the current climate chambers CO₂ concentration was held at 375±17 ppm (SD). In “future climate” chambers CO₂ concentration was within 10% and 20% of the target (620 ppm) during 84.4% and 95.6% of the time, respectively. -L17 During 4 month experiment (May– October 2007), mean monthly Tair was 14.5, 17.6, 17.4, 17.4 and 14.6. In the -3584L8-11 move to 3583L10 -L17-24 The containers were watered .. MOVE before L11 Irrigation was. -L18 start new paragraph with “Profile probes. . . -3585 L11 Fertilizer was dissolved in water MOVE to L9 4 splits when and how much water per pot? 3586L1 was separated by species L7was determined on each by multiplying N concentration -3587 L7 not necessary to repeat all data from Tab1 in the text. This makes it difficult to read. -3588L1-4. -The previous section indicated that the contrasting sensitivities of community biomass to N supply between the two climates (Fig. 1) most likely did not originate from changes in species composition. WHAT Do authors want to say here? Species composition did not change! Community biomass is the sum of all biomasses! So, changes can only arise from different contribution of species!!! WHAT WAS THE Nstock PER SPECIES??? -L4-10 NEEDS REWORDING “. the contrasting effects may arise from i) differences in N uptake (i.e. impaired uptake in the future climate at higher N supply, yielding the flat biomass-N curve for this treatment in Fig. 4) or ii)uptake was not affected by future climate but N use efficiency was. However, Figure 4 shows that N uptake was responsible, as the differences in community N stocks between the treatments mirror those in community biomass shown in Fig. 1. IS THIS TRUE??? Then authors should test this in a figure/correlation on biomass versus Nstock. I suggest to merge Fig 1 and 4 together, having biomass on the left and N on the right side to help reader. -L16-20 REWORD: ...The changes in plant N acquisition with climate might derive from altered N availability in the soil. The soil extractable nitrogen (SEN) did not differ between i) start and end of the experiment was (20.2mgNkg vs 9.2±0.3mgNkg), ii) climates nor iii) the five N treatments (Fig. 5, Table 1). This suggested that independent of climate and N supply, all communities had assimilated the same N?? -3589L4 ...attributed to changes in species compo-

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sition (?) between the – changes in biomass contribution of species -L7-12 Delete, I don not understand why authors do cite this?? They also find less responds in future climate at 1 kgN m-2 (Fig1) -L 14 This question should stand at the beginning. “What may have caused the grassland communities to respond less to N supply in a... ?” -8530 L4-8 not clear. Authors write “added fertilizer was absorbed by the plants in the future climate, but plant community N stocks nevertheless remained the same because atmospheric N₂-fixation declined. – this means that FertN replace BioFert N I am not convinced. If this is the the case authors should estimate the N-fixation of leguminous during experiment -L21 “thus enhancing the N demand of the growing and more active microbial” – I am not convinced on that either!! This would mean that the Nfert plus N mineralisation went into microbial biomass. In this case authors should probably measure soil-N by CNS and/or microbial biomass (if this is still possible) Anyhow, no NO₃/NH₄ was left in the soil at future climate. -3592 L10-14 to my opinion this should go into discussion first paragraph. -Fig 1, apply different axis scale (0-200) for Poa, Rumex and Lotus by putting them on the right side.

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