

## Call for a new proposal to combine and coordinate NutNet and Drought-Net treatments

**NPKD-Net:** A global test of combined precipitation changes and nutrient addition on plant community dynamics and functioning

**Rationale:** Changes in precipitation patterns and nutrient enrichment are two major global change drivers with strong impacts on plant community dynamics and functioning (Craven et al. 2016). The Nutrient Network (NutNet) and Drought Network (Drought-Net) are invaluable initiatives for increasing our understanding of how these drivers act independently. However, in our increasingly human dominated planet, drought events and nutrient enrichment are co-occurring and likely interacting to affect the functioning of our ecosystems. For example, in grasslands, drought events not only limit water availability to plants but also reduces their uptake of soil nutrients leading to reduced ecosystem productivity even under nutrient enrichment (He and Dijkstra 2014, Dijkstra et al. 2015, Wang et al. 2017). Understanding the generalities and contingencies of how these two drivers act in concert on grassland and heathland functioning requires a globally replicated experiment manipulating the two drivers using standardized protocols. This will further allow the identification of which climatic conditions restrict or enhance joint effects. Most studies on water-nutrient limitations in natural grasslands have *increased* water alongside soil nutrients, repeatedly revealing co-limitation (Harpole and Tilman 2007, Eskelinen and Harrison 2015, DeMalach et al. 2017). Thus, we identify the lack of drought-nutrient addition studies as a critical gap, and propose this new initiative, NPKD-Net. Given the amount of existing infrastructure of the current NutNet and Drought-Net sites, we believe the opportunity to examine such a global gradient is within easy reach.

**Call for participation:** Many participants in the NutNet and Drought-Net communities have already begun to combine the nutrient addition and drought treatments from the two networks. The first goal of this proposal is to centralize and standardize these efforts, in order to create a new coordinated distributed experiment. To this end, we have a short questionnaire attached to first ascertain how close we are to that goal at the onset. Our second goal is to attract NutNet and Drought-Net participants who have not crossed these treatments to begin doing so according to our proposed design. Finally, we encourage and welcome entirely new participants to join this initiative.

**Steering committee:** Yann Hautier (Co-PI, Utrecht University, The Netherlands), Anke Jentsch (Co-PI, University of Bayreuth, Germany), Qiang Yu (Co-PI, Chinese Academy of Agricultural Science, China), Mohammed A.S. Arfin-Khan\* (Co-PI, Shahjalal University of Science and Technology, Bangladesh), Peter Wilfahrt\* (Co-PI, University of Bayreuth, Germany), Melinda Smith (Colorado State University), Kate Wilkins (Colorado State University), Elizabeth Borer (University of Minnesota), Ashley Asmus (University of Minnesota), Eric Seabloom (University of Minnesota)

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**Data collection:** We will follow NutNet and Drought-Net protocols for data collection. This includes [first level data](#) analyses required by both protocols, and we strongly encourage collection of as many level two data as possible.

- Level one: Annual plant cover, plant IDs, biomass sorted by functional group, site characteristics.
- Level two: Litter bags, root production and biomass, light availability, plant traits, soil moisture content

**Data management:** Data will be received and processed by Mohammed Arfin-Khan and Peter Wilfahrt according to the NutNet protocol (Lind 2016). This involves receiving data from NPKD participants, standardizing data format and plant taxonomic names using repeatable R scripts while leave the raw data intact, and documenting and versioning changes to the datasets. Participants that join NPKD-Net **and** maintain a NutNet and/or Drought-Net site should send data from each experiment to Mohammed Arfin-Khan and Peter Wilfahrt. They will first receive and process the data, and then send it to Ashley Asmus (NutNet) and Kate Wilkins (Drought-Net) who will provide a final quality check and incorporate them into their respective database.

**Authorship:** We will follow NutNet and Drought-Net [guidelines](#) for authorship.

**Guidelines for Participation:** There are two ways to get involved. If you have a Drought-Net site, there are two options depending on your shelter size. If you constructed the minimum 3 x 3 m shelters, you will likely need to construct additional shelters to accommodate the new plots. However, for managed grasslands that are mown (sub)-annually you can designate a single 1 x 1 m subplot under the existing shelters for NPK addition with cover surveys and biomass harvest (Fig. 4). If you constructed larger shelters in natural grasslands (i.e. no regular disturbance), then it is possible to designate at least two 1 x 1 m subplots under existing shelters – one for cover surveys and one to three for yearly biomass harvests. You should only pursue this option if you can leave a buffer zone of at least 0.5 meters between the nutrient addition and ambient soil nutrient plots. NPK treatments should similarly be integrated into the control plots. **Any NPK additions in Drought-Net sites should NOT interfere with the core Drought-Net plots (i.e. minimum 0.5 m buffer between subplots).** For those without rainout shelters or insufficient space, we require three replicates each of ambient resources (i.e. control), drought shelters, NPK addition, and NPK x drought shelters. We propose a split plot design to minimize the number of necessary drought shelter constructions. The split plot should have a minimum 0.5 m buffer between +NPK and unfertilized plots. Each nutrient class should have four subplots of size 1.25 x 1.25 m, following the NutNet design of a permanent cover subplot with a rotation of subplots for biomass harvest (Fig. 4). This construction of rainout shelters should follow the Drought-Net protocol, which you can find [here](#).

**Additional treatments (optional):** In addition to the minimum design, we encourage participants to consider three additional treatments. 1) Addition of plots with +N in ambient precipitation and drought shelters (e.g. ambient soil resource supply, +N, +NPK) 2) A water addition treatment that is of the same severity as the drought reduction (e.g. -40% reduction, ambient, +40% addition) crossed with nutrient addition. 3) Crossing the herbivore exclosures from NutNet with the minimum design listed above.

For anyone who has already begun a crossing nutrient addition with drought shelters, please answer the brief questionnaire at the end and return to Peter Wilfahrt at [Peter.Wilfahrt@uni-bayreuth.de](mailto:Peter.Wilfahrt@uni-bayreuth.de)

Please do not hesitate to contact us ([Peter.Wilfahrt@uni-bayreuth.de](mailto:Peter.Wilfahrt@uni-bayreuth.de)) for any additional remarks or questions.

Hereafter, you will find the main hypotheses that we design to test with the NPKD-Net.

Thank you for your time and consideration.

Sincerely,

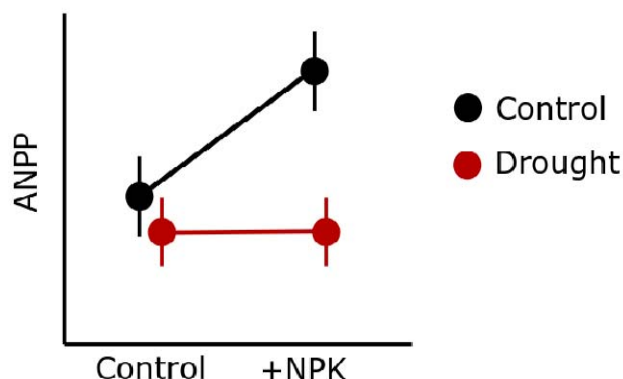
The NPKD-Net steering committee.

**NPKD hypotheses:** Similar to NutNet and Drought-Net, we envision studies covering a range of ecosystem processes emerging from this initiative. Our primary research interest is detailing the interactive effects of drought and nutrient addition on emergent plant community properties. We encourage add-on studies that further investigate mechanisms related to the joint impacts of these two global change drivers.

#### *Productivity hypotheses*

Biomass production is often co-limited by multiple soil nutrients (Fay et al. 2015). However, the response to nutrient enrichment is dependent on the natural precipitation regime of the site (Wang et al. 2017). Xeric systems, being already water limited, may be less likely to show a response to NPK addition under drought (Morgan et al. 2016).

- We expect to observe an interaction between drought and +NPK addition, where drought nullifies or reduces the +NPK effect (Fig 1).
- We further expect the relative magnitude of this interactive effect to be stronger at more xeric sites, and may detect a threshold for where this interaction begins along a precipitation gradient.

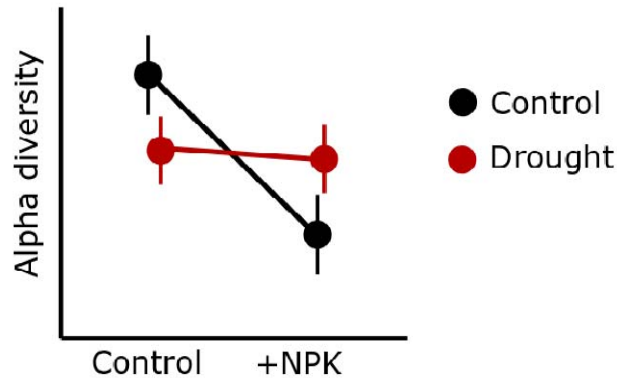


**Fig. 1** – Conceptual figure of central NPKD-Net ANPP hypothesis. Nutrient addition effects are contingent on sufficient water availability.

#### *Diversity hypotheses*

Soil resource limitation maintains community diversity, and nutrient enrichment leads to species loss (Harpole et al. 2016). Drought may lead to local loss of drought sensitive species such as legumes (Eskelinen and Harrison 2015). However, immobilization of soil resources during drought may moderate the loss of species during nutrient enrichment.

- We expect drought to suppress the competitive exclusion that typically leads to decreased community diversity following nutrient addition (Fig. 2).
- Traits conferring drought resistance and growth responses to nutrient enrichment of the dominant species at each site will determine relative sensitivities to drought, NPK addition, and their interactive effect.



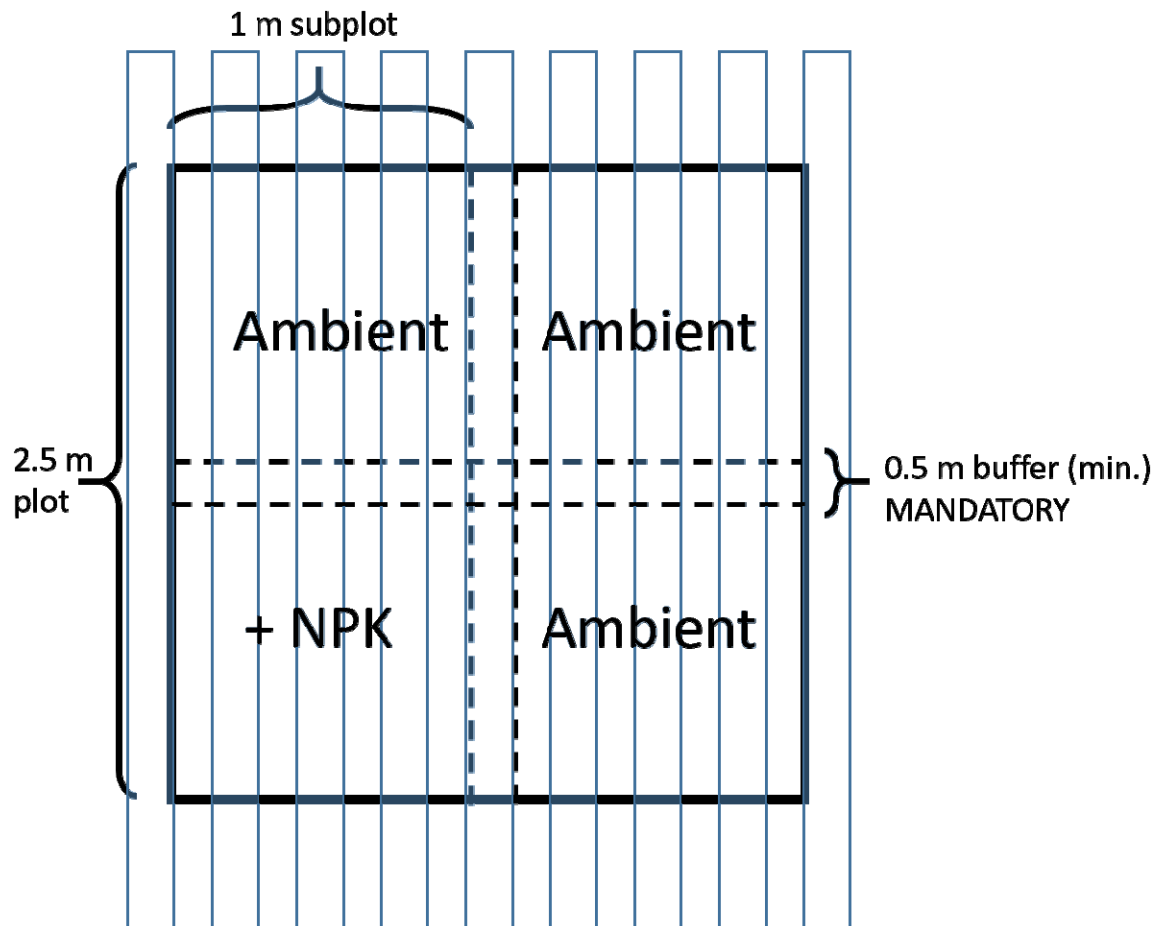
**Fig. 2** – Conceptual figure of central NPKD-Net diversity hypothesis. Losses of species diversity after nutrient addition effects are greater when water is not limiting.

#### *Diversity-biomass-stability hypotheses*

Nutrient enrichment should favor ‘fast’ species with exploitative strategies (rapid resource uptake, growth and tissue turnover) at the expense of ‘slow’ species with conservative strategies (slower rates of growth, resource uptake and tissue turnover). The leaf economics spectrum (Reich 2014) predicts that slow communities should have traits that confer greater resistance against drought, while fast communities should have traits that confer greater resilience. The net effect on the temporal stability of the ecosystem is determined by the combination of the resistance and resilience (Isbell et al. 2015).

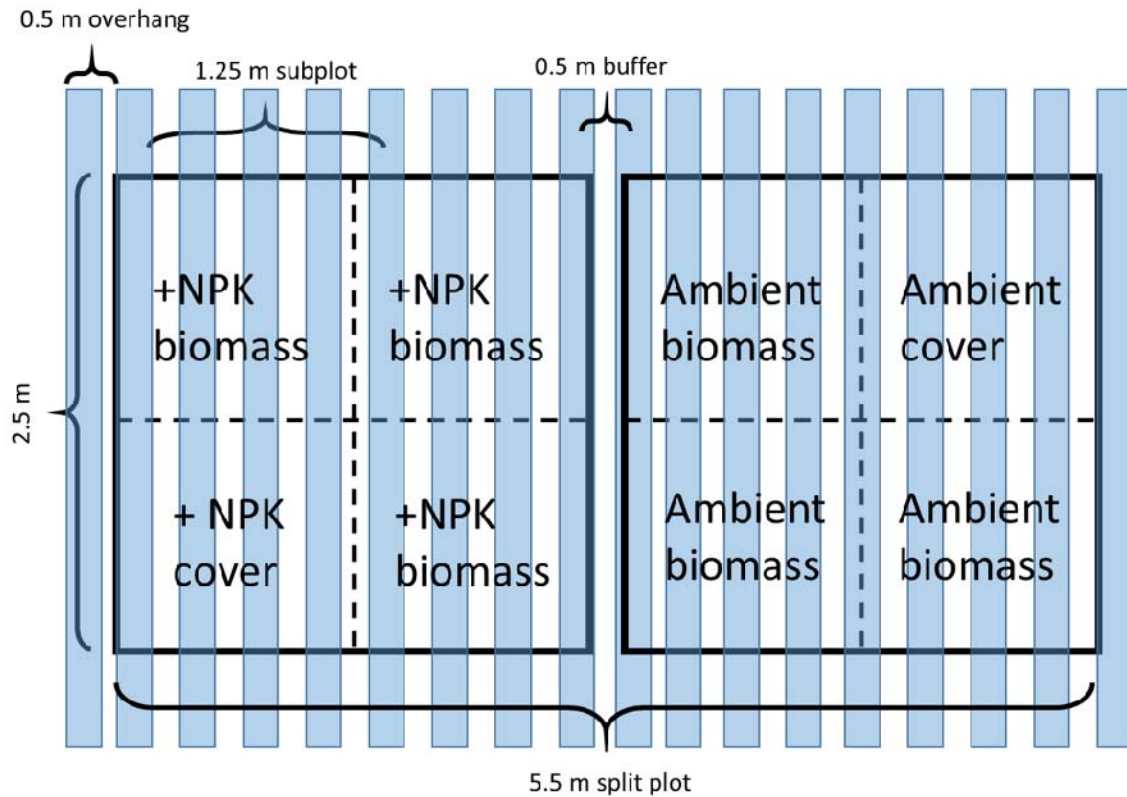
- Communities composed of ‘fast’ and ‘slow’ strategy species should be more stable in biomass production through time due to a combination of resistant and resilient species with asynchronous responses to climatic variability.
- Nutrient enrichment will reduce the stability of biomass production through time due to selection of fast species with low resistance but high resilience and thus less asynchronous responses.
- Species rich communities will be naturally more stable due to a higher likelihood of including a combination of resistant and resilient species.
- Xeric systems will better maintain stability due to already water-limited conditions and decreased responses to NPK addition under drought.
- These effects might be weaker through time due to adaptation.

Example Drought-Net design for 3 x 3 m shelters in managed grasslands

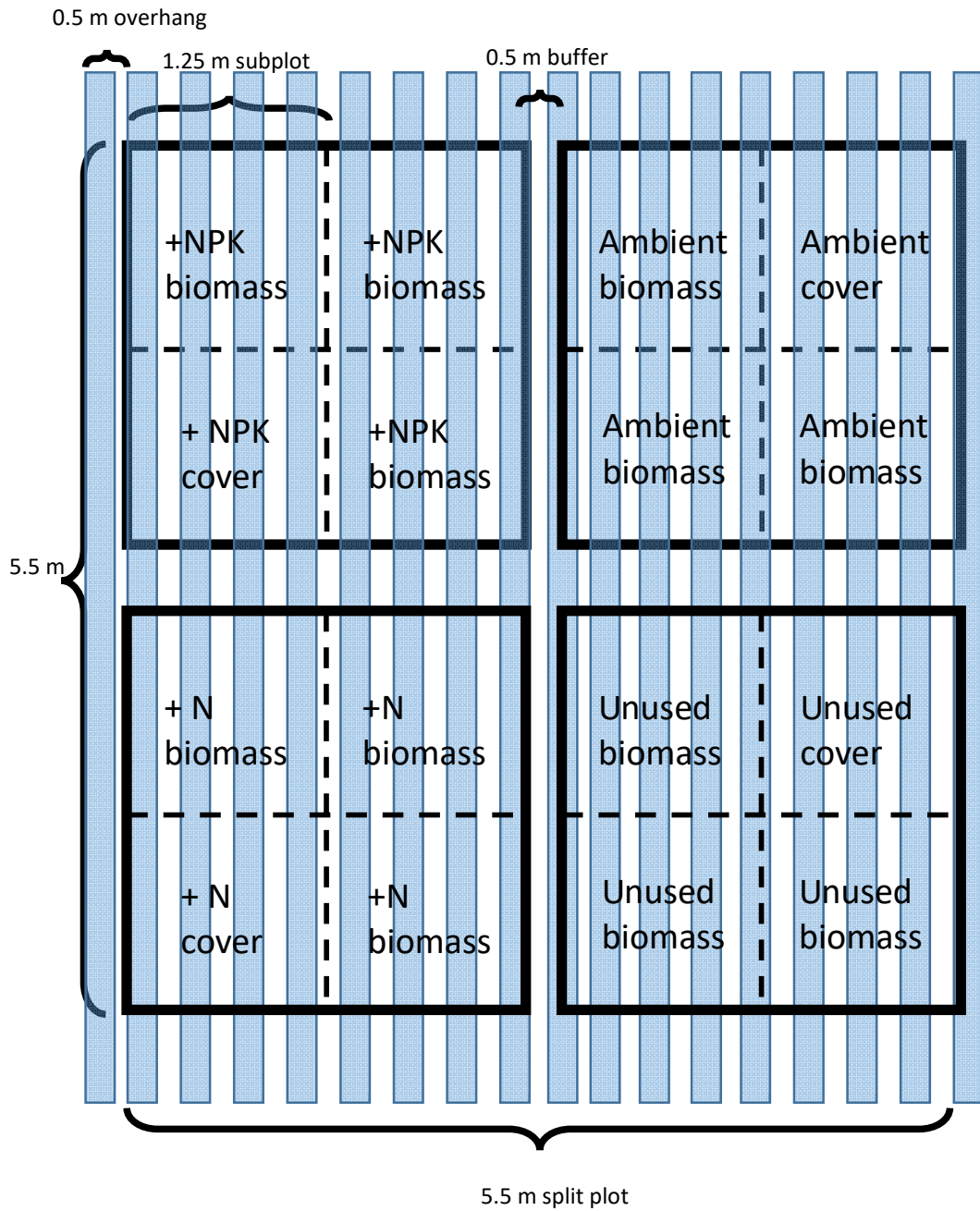


**Fig. 3** - In Drought-Net plots where biomass is removed yearly, NPK can be added to a subplot where both cover is recorded and biomass is harvested provided a 0.5 m buffer can be maintained. In unmanaged grasslands, addition of nutrients should only be added in new shelter constructions, or in larger, existing rain shelters that have a big enough footprint to accommodate nutrient addition without infringing on the core Drought-Net activities.

**Example split plot design for new NPKD plots**



**Fig. 4** - For existing NutNet sites, Drought-Net sites with insufficient space (see above), or new sites altogether, we propose a split-plot design. This would require the construction of three rainout shelters built using the Drought-Net specifications that can accommodate a 2.5 x 5.5 m plot, meaning a 3.5 x 6.5 m footprint of the rainout shelter. This would be split into two sides, one side receiving the +NPK treatment and the other left at ambient soil nutrients. They would further be split into subplots that allow for annual cover data collected in the same area along with three subplots where biomass can be harvested in rotation. In a block design, these would be paired with a split plot with no rainfall reduction.



**Fig. 5** – Example of expanded nutrient addition plots. Here, the drought shelter is extended compared to Figure 4 to allow additional nutrient treatments.

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### **Questionnaire for sites already crossing nutrient addition with drought treatments**

- 1) Please provide country, site name, PI(s), contact email address
  
- 2) What year did you begin the crossed treatments?
  
- 3) Please describe the experimental set up (e.g. plot size, number of replicates, block design, etc.)
  
- 4) Please briefly describe the data you are collecting (e.g. annual cover, biomass sorted by functional group, etc)
  
- 5) Please briefly describe the nutrient treatment you are crossing with drought (e.g. +NPK only, Full factorial design of NutNet, etc.)
  
- 6) Please briefly describe the drought treatment(s) you are crossing with nutrient addition (e.g. Drought-Net design, full rainout shelter, irrigation etc.)
  
- 7) Did you incorporate the fencing treatment of NutNet into the design? If so, please describe.
  
- 8) If necessary, are you willing to modify your design to match NPKD-Net?
  
- 9) If you maintain both a Drought-Net and NutNet site, do they share the same control plots?