# Project: effect of nutrient availability on flower resources, pollinators and pollination

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**Summary:**

Several studies have looked at impacts of increased nutrient availability on plants. However, there is still little information on how such impacts affect plant-pollinator interactions. This project aims to cover this gap of knowledge by collecting data on flower abundance, flower visitation, pollinator contribution to fruit set and overall fruit set in plant communities exposed to different fertilization treatments in different parts of the World.

Preliminary results from the Brazilian Nutnet site (Três Lagoas) show that nitrogen (N) fertilization stimulated flower production and pollinator visits to flowers (bees, butterflies), although N fertilization did not affect biomass production or light conditions. However, the opposite was found in a long-term fertilization experiment in Brussels, where N addition has strongly increased biomass production, but diminished light penetration to the soil as well as density of insect-pollinated flowers (Abts, 2018). Phosphorus (P) or potassium (K) fertilization did not have significant effects on flowers or pollinators in both experiments. Hence, in both experiments the impact of nutrients on plant-pollinator interactions varied strongly among the different nutrients, and depended on local (growth-limiting) conditions. Greater coverage of climatic regions and plant species is needed to understand how local conditions (e.g. plant community composition, growth-limiting conditions) might be regulating the effects of nutrients.

Using different sets of data (A-D, see below), this proposal aims to answering the following questions in a Nutnet study:

- Is the effect of increased nutrient availability on flower production (part A) , pollinator visits (Part B) and fruit production (part C and D) mediated by local soil conditions?
- Does the effect of nutrients on flower resources (part A) depend on plant's growth response?
- Does the absence of large herbivores (excluded with fences) affect insect-pollinated flower production (Part A), pollinator visits (part B) and fruit production (part C& D)?
- Is the effect of increased nutrient availability on flower production (part A), pollinator visits (Part B) and fruit production (part C and D) mediated by climatic conditions?

Participating sites can collect data on all steps of the nutrient-flower-pollinator-fruit chain, or participate in only the less time-consuming parts (e.g. parts A and D which only demand a minimum of one 1day survey during the main flowering or fruiting season, see below).

All contributions, of as many Nutnet sites as possible, are very helpful for this project. We consider parts A and D as baseline input to understand impacts on resources used by flower visitors. Information on B is an important addition to help understand how pollinators react to the changes detected in A and mediate the effects detected in D. Part C is more time consuming and can be seen as 'icing on the cake', since it would allow to disentangle the direct effects of fertilizers on production, from those mediated by pollinators. The more sites participating in C and D, the more substantial our conclusions can be about fertilization effects on plant-pollinator interactions on a global scale. Therefore, we encourage the Nutnet community to find ways (e.g. student projects?) in participating not only in the baseline parts but also in the more time-consuming parts.

# Protocol for plant-pollinator data in NutNet plots

For all sections below we propose to focus on the NutNet treatments: Control, N, P, K, NP, NPK. Additional data on NK, PK, fenced control and fenced NPK, would also be interesting.

### *A. Flower abundance*

Flower abundance in each plot should be estimated immediately before or after flower visitation surveys (see below). Nutnet sites that only participate in part A (or only A and D) should measure flower abundance at least once during the peak of the flower production period.

In each survey, and for each flowering plant species (wind pollinated plants, such as grasses, can be excluded), all flower units within the plot (5x5m) are counted. If for some species flower abundance is very high an estimation of total flower unit number can be done based on randomly selected subsamples (e.g. ten samples based on a 0.5m2 quadrat). **This part of the work takes approximately 15min per plot.**

A floral unit was defined as approximately 1 cm2 with at least one open flower (Carvalheiro *et al*., 2014). For some species, such as *Portulaca amilis*, 1 floral unit corresponds to 1 flower, for certain members of the Asteraceae such as *Praxelis pauciflora* and *Vernonanthura chamaedrys*, 1 floral unit corresponds to 1 inflorescence, while for *Waltheria indica* 1 floral unit corresponds to a flower bud containing 1 to 5 flowers (Fig1.).

Date, time and local climatic conditions (temperature, humidity and wind speed) should be recorded/measured ideally locally (with a portable weather station) or extracted from climate websites with information on local weather conditions

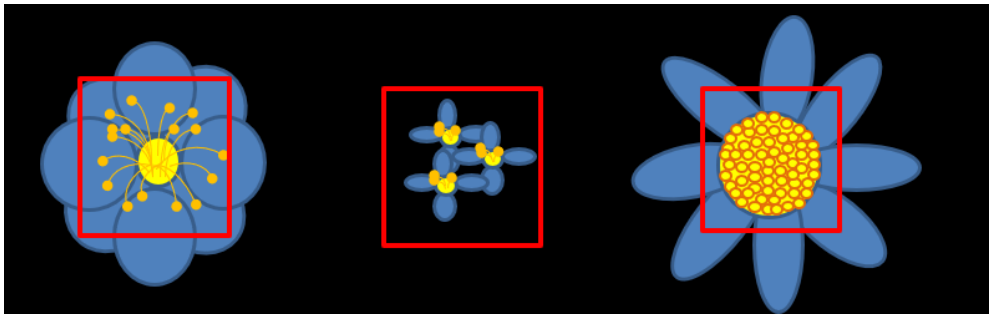
Figure 1. Examples of flower units, defined as 1cm2 with at least one open flower.

***B. Flower visitation surveys***

Observational flower visitation surveys should take place on days with sunny to partly cloudy conditions, without strong winds.

In each survey, each plot should be observed for 20 minutes, between 8AM and 11AM, and again between 13PM to 16PM. Several plant species can be observed simultaneously, but if insect activity is very intense, it will be advisable to observe only one or two species simultaneously. T**his part of the work can take 20-60min per plot depending on insect activity.**

Ideally all flowering species within all plots selected for this experiment should be observed within a total time period of 5 to 10 days.

The sampling protocol suggested below follows the guidelines FAO protocol to detect and monitor pollinator communities (http://www.fao.org/3/a-i5367e.pdf), which was designed to be applied by non-specialists. In order to minimize disturbance of flower visitors and maximize detection of visitor activity, the surveys should performed by walking very slowly (about 0.5 m per second) around the plots. Whenever potential visitor activity was detected in the center of the plot yet not visible enough from the sides, a few steps towards the center of the should be made carefully. A flower visitation event is defined as an insect making contact with the reproductive parts (stigma, anthers) of the flower, thus disregarding those insects that were sitting on the outer petals obviously not feeding on nectar or pollen. In doing that, we increase the likelihood of registering only pollination events.

Once a flower visitation event is observed, the visited plant species is registered, and insects are caught with a net and collected in killing tubes. If the insect escapes, information of the order (if known) and a morphological description of the visitor (e.g. colour & size) should be registered.

All collected morphospecies should be mounted and sent to expert taxonomists, if possible. All uncollected visitors will be considered for abundance analyses. However, those will only be considered for visitor richness analyses if the description of an escaped specimen does not match with any of the collected morphospecies.

For sites where flower visitation surveys can be repeated monthly, we will also be able to assess changes in phenological patterns (see Biederman et al. 2017)

### *C. Pollinator contribution to fruit set survey*

This part can be focused on a few plant species (e.g. top 5 most abundant species) that occur at least in two control plots and two plots where nutrients have been added.

To evaluate the effective contribution of flower visitors to plant reproduction (i.e. pollination), for each plot and selected plant species, 10 flower units belonging to at least three individual plants should be selected before anthesis (i.e. when flowers are still immature, buds) and marked. Five of those will be left as control, and the remaining five should be bagged with a fine mesh (1mm), to exclude all flower visitors. The bag should be placed so that they do not touch flower reproductive parts (depending on the plant species wire rings may help to keep the bag in the right position, see NSF site for a visual example: https://www.nsf.gov/news/mmg/mmg_disp.jsp?med_id=75637&from= ). After flower anthesis finishes bags should be removed. Fruit set should be accessed in each of the marked flowers, ca. 1-2 month(s) after (exact time period depends on plant species characteristics).

This part of the work requires three full days of field work, one for setting the exclusion bags, another for removing the bags after anthesis, and another for counting fruits.

### *D. Overall fruit set evaluation*

For all plant species of each plot, fruit set survey should be done ca. 1-2 months after flower visitation surveys, by walking slowly around and within each plot and counting all fruit units. For species with very high flower abundance an estimation of total fruit unit number can be done based on randomly selected subsamples (e.g. ten samples based on a 0.5m2 quadrat). A fruit unit can be defined by data collector as a single fruit (e.g. Rosaceae) or a infructescence (e.g. Asteraceae).
T**his part of the work takes approximately 15min per plot.**

## References

Abts, L. (2018). Fertilization drives the abundance of flowers and their visitors. MSc Thesis, Vrije Universiteit Brussel. Brussels, Belgium.

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