Design of a factorial *N × P application* experiment

(Please consider: the main limitation of the PxN application experiment is that only short-term effects can be analyzed. However, this approach provides a promising tool to investigate the ecosystems' response to increased P and N concentrations and to changes in N/P ratio at the different sites with different P status. If you wish to study long-term P fertilization effects (available on P poor sites only), there would be the possibility to consider the Oerrel long-term fertilization experiment coordinated by Dr. Ulrike Talkner. If you are interested in the Oerrel experiments please contact us. We offer to coordinate the activities at this site.)

Hypotheses - *P addition*:

(1) *Fate of applied P*: Phosphate applied to the soil surface is used more efficiently on recycling sites compared to acquiring sites. High use efficiency is achieved by (1) higher uptake rates by plants and microbes in the forest floor, (2) efficient cycling within plants (3) P storage in living biomass, (4) P storage in soil organic matter, (5) less leaching from the forest floor to the mineral soil, and as a consequence less fixation by the mineral soil compared to acquiring sites.

As an alternative response a shift from recycling to acquiring systems might be expected:

(2) *P concentration as the key for P strategies*: P addition induces the shift of P-recycling systems to P-acquiring systems.

***N addition*:**

Continuously high concentrations of inorganic N disturb efficient recycling of P. Possible mechanisms: On recycling sites (poor in P) additional N increases microbial activity (as microbes are no longer N limited) and therefore the P demand of microbes increases. This process enhances the mineralization of soil organic matter and the leaching of P as DOP. On P rich sites the application of N fertilizer reduces the need to mobilize N from soil organic matter, while P is derived from the mineral P-pool of soils. Thus, N addition leads to an accumulation of soil organic matter on P rich sites.

Study sites: BBR > MIT > LUE

General experimental design (see fig.1):

- Treatments: Control, P addition, N addition, N+P addition
- 3 replicates of 4 randomly distributed plots, each of them 20 x 20 m (= 12 plots per site)

- *Position of plots: Relocation of nutrients from fertilized plots to other plots has to be avoided (slope!!)*
- One quarter of each fertilization plot will be treated with isotopically traced fertilizers: ^{15}N,
  → *greatest possible distance between isotopically labeled subplots*
- Addition of ^{13}C labeled litter is intended
- each plot will include at least two beech trees (120 years) and natural regeneration (0-10 years)
- minimal distance between different plots: 20 m
- instrumentation of fertilization plots: ion exchange resin tubes; additional gas flux measurements during application of fertilization

Amount and form of applied nutrients:

150 kg N /ha as Ammonium nitrate (NH_4NO_3); dosage: 5x30 kg N ha (4x2016, 1x2017)
50 kg/ha P as Potassium dihydrogen phosphate (KH_2PO_4); one application in May 2016
(If only small effects are observed after addition, additional smaller application will be performed during 2017 and/or 2018)
KCl will be applied to control sites and N-application sites to account for K-addition to P-plots.

→ Application in dissolved form with garden sprayer

Timeline:

October/November 2015:	Selection of all plots
November 2015 – April 2016:	Analysis of pre-application samples
April/May 2016:	Application of P fertilizers and N fertilizers (first dose)
Further N-Application:	June, August, Oktober 2016 and May 2017

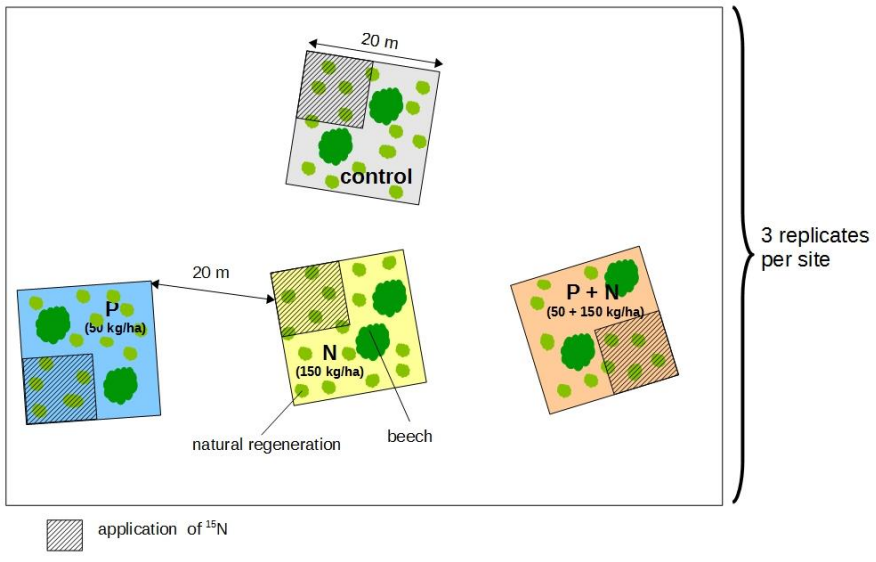

Figure 1: *Experimental design for the NxP application experiment*

Girdling experiment

Girdling of beech trees will provide insights into the competition between trees, fungi and bacteria for P. The following hypothesis will be tested:

Hypothesis:

On P-poor sites girdling will increase Pmic and microbial turnover due to reduced P uptake by plants (reduced competition). On P-rich sites girdling will reduce Pmic and microbial turnover due to reduced assimilate input from plant roots. Hypothesis/Expectation: in bulk soil C-limitation → no significant effect of girdling; rhizosphere no C-limitation → significant effect e.g. on microbial community → indirect effect on P nutrition

BBR and LUE: 4 additional smaler plots per site with girdled beech trees (10 years)

- plot size: 5x5 m or even smaler
- plots surrounded by trenches
- control girdling (may be control from NXP-experiment)
- Only for short period (e.g. 3-4 month, June – September/October)
- Proposed start: **spring 2017**