**Climate Change Impairs Nitrogen Cycling in European Beech Forests**

**Short Title: Nitrogen cycling in intact beech-soil systems**

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**Supporting materials and methods: “Analysis of distribution of beech forests on calcareous soils in Europe”**

The map (Figure 1) is an intersect of a species distribution model for European beech [1] based on maps of geology for Europe. For the species distribution model we used a large dataset of presence/absence information for European beech derived from the ’Data on Crown Condition of the systematic grid (16 x 16 km)’ (Level I) from the ’International Co-operative Programme on Assessment and Monitoring of Air Pollution Effects on Forests’ (ICPF) [2] as response variable. The dataset with overall more than 8000 plots contains 1097 presence values for Fagus sylvatica. This response was modelled using derivations of precipitation and temperature from the WorldClim database as described in reference 3. For the initial model we used nineteen bioclimatic variables included in the database and an additional calculated set of six bioclimatic variables consisting of the number of days per year with mean temperature above 5 degree Celsius, the yearly heat sum above 5 degree Celsius, mean temperature and precipitation sum in summer (defined as the months May to September) and annual and summer drought index according to reference 4. For the projection under future conditions, we used output from the global circulation model HADCM3 [5] driven by the SRES scenario A2 [6] until the year 2080, which was calibrated and statistically downscaled to 30-arc-second tiles using the WorldClim data for ’current’ conditions.

As a statistical model formulation we fitted a Generalized Linear Model (GLM) with logit link functions, (i.e. logistic regressions). We used second-order polynomials of the described bioclimatic variables on the link scales of the GLM and reduced the number of the predictors using the sum of the adjusted deviances as a statistical measure. We calibrated the model by a stepwise selection checking for changes in the Bayesian Information Criterion [7]. The final five bioclimatic variables that entered the model in linear and quadratic form were: yearly sum of degree days above 5°C, iso-thermality, drought index after O’Neill et al. [4] over the year, sum of precipitation in the warmest quarter of the year and the precipitation of the most humid month. As the threshold value for presence or absence we used Cohen’s Kappa [8].

S1 Figure shows the histograms of predicted probabilities (upper left), the observed prevalence plotted against the mean predicted probabilities by probability classes (upper right), the ROC curve (lower left) and the threshold-dependent accuracy measures (lower right) for the modelled species European beech (Fagus sylvatica). The histograms of the predicted probabilities show a maximum at p= 0.0 for absence and between 0.4 and 0.5 for presence. The predicted probabilities are well distributed along the diagonal (upper right panel) and both, area under curve (AUC) of the receiver operating characteristic with a value of 0.86 (lower left panel) and (following[9]) Cohen’s Kappa with a maximum value of 0.43 (lower right panel) indicate good performance of the model.

To validate the model we carried out a 10-fold cross -validation. Therefore, we randomly split the data into a training and an evaluation dataset [10] and used 75% of the data records for training and the remaining 25% for evaluation [11].

S1 Table shows some statistical indices of the 10-fold cross-validation. The adjusted deviance was calculated according to [10] the displayed threshold maximizes Cohen’s Kappa. Clearly, the model appears to be robust with the cross validation means being very close to the model values, the variation within cross validation results being low and the cross validation results displaying not a single outlier.

A detailed description of the modelling approach, the choice and selection of the predictors and the database of the model used for this investigation can be found in reference 1. For a general overview on species distribution models for major tree species in Europe see also reference 12, specifically in the supplement of this publication.

Using the described model we produced two maps depicting the potential distribution of European beech under current climate (1950-2000) and for scenario A2 until the year 2080 and intersected these maps with maps of the geology of Europe [13]. In these European maps with a scale of 1: 5'000'000 [13] we selected all pixels that were assigned to formations including calcareous, limestone or other basic substrates. For Germany where no detailed information on the substrate was displayed in the European map, we used the 1:1'000'000 map for the Geology of Germany [14]. For areas in Europe such as Poland, Estonia, Lithuania and Latvia that did not display information on Geological map of Europe as well, we examined whether European beech plays a significant role in the species distribution. As this was not the case for both time periods we excluded these parts of Europe from the analysis. The intersected maps were created using standard overlay functions.

**Supporting materials and methods: “Calculation of N pools, isotope recovery and gross rates of N turnover”**

Measurements of N pool size in soil and plant as well as isotopic information of plant, soil organic, inorganic, microbial and mycorrhizal N pools was used to calculate gross rates of N turnover and isotope tracer recovery.

**Calculation of soil dry weight**

sdw : soil dry weight (g)

sfw : soil fresh weight in the mini lysimeter (g)

D/W : quotient between dry soil after drying at 105°C and field fresh wet soil

**Total extractable soil N pool sizes**

**Ammonium-N pool (14+15) [µg N/g sdw]**

concNH4+ : concentration of NH4+ in soil extract (mg N l-1)

BW = blind value -N in K2SO4

= volume 0.5M K2SO4 (ml)

**Nitrate pool (14+15) [µg N/g sdw]**

**Dissolved organic N pool (14+15DON) [mg N/kg sdw] calculated as TNb-DIN**

TNb: total chemically bound N in soil extracts (mg l-1)

DIN: dissolved inorganic N in soil extracts (mg l-1)

**Microbial biomass nitrogen** **(MBN)** **[mg N kg-1 sdw]; (X)fum= data from chloroform fumigated soil**

**Plant pool (P) [mg N mesocosm-1]**

pdw=total dry weight of plant tissue (mg)

Information on soil and plant N pools was transferred to the unit mg N m-2, considering the dry mass of soil contained in a beech-soil-mesocosm and/or the surface area.

**15N excess amount**

**, , DON [mg N kg-1 sdw]**

[\*X]

[\*X]= 15N excess amount (mg N kg-1 sdw) with X = , or DON

[X]: N concentration in pool X (mg N kg-1 sdw)

[%X]: Atom% 15N enrichment of pool X

Nat abund: natural abundance 15N atom% excess (unlabelled soil).

**15N excess amount in microbial biomass** **(MBN) [mg N kg-1 sdw]**

**15N excess amount in plant biomass [mg N lysimeter-1]**

The recovery of 15N excess added by labelling was calculated by dividing the 15N excess amount detected in the investigated pools by the original amount of 15N excess added through the labelling solution.

**Calculation of gross rates of N turnover**

Gross rates of ammonification and nitrification were calculated following the 15N pool dilution equations given by [15].

**Ammonification [mg N kg-1 sdw day-1]**

Subscript [ ]: Measurement time (0 = before labelling; 6 = 6 hours after 15N application; 48 = 48 hours after 15N application)

**Nitrification [mg N kg-1 sdw day-1]**

Plant N uptake and microbial immobilization of inorganic N was calculated based on short-term tracing (6 hours) of labelled substances (15NH4+, 15NO3-) into plant biomass. The short period was chosen to minimize bias due to unquantified tracer outflow from the sink pools and depletion of tracer in the source pool[16, 17].

**Plant uptake [mg N lysimeter-1 day-1]**

[APE]: Atom% excess of pool X

= - N atomic % excess immediately after 15N labelling

- N atomic % excess six hours after labelling.

**Plant uptake [mg N lysimeter-1]**

**Microbial inmobilization [mg N kg-1 sdw day-1]**

**Microbial immobilization [mg N kg-1 sdw day-1].**

All N turnover rates were transferred to the unit mg N m-2 day-1, considering total dry soil contained in the beech-soil-mesocosm and the surface area. Plant uptake rates were transferred to the same unit by considering the surface area of the beech-soil-mesocosm.

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