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Immobilisation of nitrogen in context of critical loads

Literature review and analysis of German, French and Swiss soil data

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Immobilisation of nitrogen in context of critical loads

Literature review and analysis of German, French and Swiss soil data

by

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Kurzbeschreibung

Im Rahmen der Critical Loads Berechnung ist die bestmögliche Abschätzung der Stickstoff-Immobilisation unter steady-state Bedingungen ein wichtiger Faktor. Zur Zeit sind verschiedene Ansätze in der Diskussion. Daher wurde eine Literaturstudie zu vorliegenden Immobilisationsraten und Einflussfaktoren ebenso wie eine Abschätzung anhand von Inventurdaten aus Frankreich, der Schweiz und Deutschland durchgeführt. Für letztere wurden die Immobilisationsraten anhand von Stickstoffvorrat und Alter der Böden berechnet.

Die Werte der langfristigen Stickstoff-Akkumulationsraten in der Literatur beruhen auf Input-Output-Bilanzen bzw. auf kurzfristigen Veränderungen der Stickstoffvorräte im Boden. Diese aktuellen Raten reichen von 1.8 und 42 kg ha-1 yr-1. Es werden sogar negative Werte beschrieben. Die Werte variieren mit der Beprobungstiefe und können von innerhalb eines Profils von Immobilisation zu Mobilisation wechseln. Die in der Studie berechneten N-Immobilsationsrate sind niedriger. Der Mittelwert der N Immobilisationsraten für glazial geprägte Standorte reicht von 0.4 kg ha-1 yr-1 in Deutschland und 0.8 kg ha-1 yr-1 für die Schweiz nd Frankreich. Unter der Annahme der periglaziale Böden ca. 24.000 Jahre alt sind ergeben sich Werte von 0.035 bis 1.6 kg ha-1 yr-1 mit einem Mittelwert von 0.2 kg ha-1 yr-1 für Frankreich und Deutschland sowie 0.4 kg ha-1 yr-1 für die Schweiz. Dies ist eine Abschätzung und überschätzt die Rate , wenn Böden älter sind als 24.000 Jahre. Trotzdem zeigt es , dass die Immobilisationsraten in der organischen Auflage bis 60cm geringer als 1.5 kg ha-1 yr-1 (90 Perzentil). Diese Ergebnisse decken sich sehr gut mit jenen von Rosen et al. (1992) und Johnson & Turner (2014), die eine jährliche Rate von zwischen 0.2–0.5 kg ha-1 yr-1 und 0.5–1.0 kg ha-1 yr-1 angeben. Einen Zusammenhang zwischen Temperatur und N-Vorräten konnte nicht gefunden werden, wohl aber mit der mittleren Lufttemperatur und der Höhe. Der höchste Korrelationskoeffizient ergab sich zu C-Vorräten.

Auf Grundlage der Auswertungen zeigte sich, dass die stratifizierten N-Vorräte bis 40 cm geeignet sind, die langfristigen N-Immobilisationsraten im Rahmen der Critical Loads Berechnungen abzuschätzen. Die Daten liegen in den internationalen Monitoringprogrammen für viele Länder vor bis 40cm.

Abstract

Estimating nitrogen immobilisation under steady state conditions is an important part in the Critical Load computation. Different approaches are available and currently under discussion. A literature review of immobilisation rates and impact factors had been done as well as an evaluation of nitrogen stocks from soil inventory data of France, Germany and Switzerland. We estimate immobilisation rate by soil age and nitrogen stocks.

The results of a literature review of long-term N accumulation rates mentioned above are contradicted by results of input-output budgets and studies of short-term changes in soil N stocks. This current N accumulation rates can show positive expression with rates between 1.8 and 42 kg ha-1 yr-1 as well as negative rates. Changes in N stocks often vary in different soil depths and furthermore can change from accumulation in one layer to mobilisation in another. The calculated immobilisation rates from inventory data are quite lower. The median of N accumulation rates of glacial sites are 0.4 kg ha-1 yr-1 for German and 0.8 kg ha-1 yr-1 for Swiss and French sites. Assuming that periglacial sites are at least 24000 years old the N accumulation ranged from 0.035 to 1.6 kg ha-1 yr-1 with a median of 0.2 kg ha-1 yr-1 for France and Germany and 0.4 kg ha-1 yr-1 for Switzerland. This calculation is very rough and is an overestimate in cases where soils were much older than 24 000 years BP. Nevertheless, this calculation suggests that N immobilisation in OL and mineral soil till 60 cm soil depth is less than 1.5 kg ha-1 yr-1 (90 percentile). Our results are consistent with Rosen et al. (1992) and Johnson and Turner (2014) who estimated the annual N immobilisation since the last glaciation at between 0.2–0.5 kg ha-1 yr-1 and 0.5–1.0 kg ha-1 yr-1, respectively. The results of the correlation analyses show no signifi-

cant negative correlation between N stocks and mean air temperature in all soil compartments. In contrast N stocks are significant positive correlated with mean annual precipitation in all compartments the altitude and the N stocks are significant positive correlated with correlation coefficients between 0.009 and 0.25 (not significant in TS 60cm). The strongest significant correlation with coefficients between 0.76 and 0.98 was found between N stocks and C stocks.

According to the stratified N stock analysis the upper layers of the mineral soil contains the major part of the total soil N. In order to estimate the long-term N immobilisation for the Critical Load calculation these top soil layers (40 cm) might be sufficient. This would comply with most international monitoring programs, which are limited to this depth.

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List of Abbreviations

MAT	mean annual temperature
N	nitrogen
NH_4^+	Ammonium
N_i	Nitrogen immobilisation
SOM	soil organic matter

1 Introduction

Durable nitrogen immobilisation (N_i) is one of the least well documented parameters of the simple mass balance equation inputs but is crucial to determining an accurate critical load (Bingham & Cotrufo 2016). There exist various definitions of N_i, with differences in including processes (abiotic, biotic) and in reference depth (organic layer, topsoil, root zone). Therefore it is necessary to establish a consistent terminology as a requirement for calculation of N_i. In context of critical loads, N_i is the long-term net immobilisation or accumulation of nitrogen in the root zone without changes in the prevailing C/N ratio (ICP 2017).

In first part of our paper we represent the result of literature review and going to answer following questions:

- Which definitions of N_i exist and what are differences between them?
- What processes contribute to long-term accumulation of nitrogen in soil?
- Which parameters influencing long-term N_i?
- Which values for long-term accumulation of nitrogen in the soil can be found in literature?

The second part of this paper deals with the amount and distribution of total nitrogen (N) in organic layer and mineral soil of forest ecosystems in France, Switzerland and Germany. We estimate accumulation rates for nitrogen by dividing total N stocks by the expected age of the soil, recommended by Grennfelt (1992). All results are restricted to forest ecosystems.

2 State of the Art – Results of literature review

Literature research was done by using the databases of Web of knowledge and Google Scholar. To find adequate papers we used the keywords: nitrogen (-accumulation, -immobilisation, -retention, -budget, - transformation, - turnover, - sequestration, -stabilization, -incorporation, -chronosequence and -succession). We focused on literature representing data of forest ecosystems, published between 2004 and 2017. Furthermore older studies representing data from soil chronosequences were additionally included.

2.1 The processes of nitrogen immobilisation

The process where inorganic nitrogen is transformed into an organic form by incorporation into microbial biomass (free-living or mycorrhizal) is commonly known as biotic immobilisation. Thus microorganisms transfer N into the soil organic matter (SOM) by creating N-containing necromass and excreting N-containing exoenzymes (Lewis & Kaye 2012). Furthermore the term N immobilisation involve an abiotic process where ammonium (NH_4^+) can be fixed in clay lattice or can be bound to organic matter (Young & Aldag 1982; Stevenson 1994). It's assumed that abiotic immobilisation play a subordinated role, because more than 90 % of nitrogen in soil is organic (Stuhrmann 2000; Blume *et al.* 2002; Nannipieri & Eldor 2009). Nevertheless in soils with a notable amount of ammonium-fixing clays inorganic nitrogen can reach up to 25 % of total soil nitrogen (Blume *et al.* 2002). Johnson and Turner (2014) supposed that nitrogen almost never accumulates in soils in inorganic form for any length of time.

In case of critical loads the term N immobilisation is used in another context which is different to the definition mentioned above. N_i represents the amount of N which is retained long-term in the root zone of soils by build-up of stable C-N-compounds (ICP 2017). Long-term N accumulation, e.g. the net immobilisation in soil is assumed to be very slow and takes serval hundred years (Nilsson & Grennfelt

1988). The prevailing C/N ratio of soil should not be change significantly through this long-term N immobilisation (ICP 2017).

Both definitions refer to different soil processes, but there is no retention of N without microbial immobilisation. Nearly all N accumulated for long-term has undergone microbial processing (possibly many times over) before it is removed from circulation. Thus microorganisms exert an important influence in regulating the amount of N suitable for long term storage (Knicker 2011). Due to short microbial lifetime the microbial incorporated N is continually transferred within the soil from organic to inorganic forms and back again (mineralisation-immobilisation-turnover). The mean residence times for microbial N in organic layer and topsoil ranging from 8 to 32 days (Corre *et al.* 2007) and 52 days (Kreutzer *et al.* 2009). The C and N chemistry of the substrate, C and N status of the soil, the structure of microbial community and the edaphic and environmental factors (temperature, moisture, porosity) affect the efficiency and quantity of microbial immobilisation (Bingham & Cotrufo 2016).

But there is still a lack of understanding which mechanisms are involved in soil organic nitrogen stabilization (Knicker 2011). The adsorption of N-containing compounds onto mineral surfaces is supposed to be one process which contributes to long-term accumulation of nitrogen. The movement of adsorbed N is retarded within the soil, thus other retention mechanisms, such as microaggregate formation and spatial separation from microorganisms become more effective (Bingham & Cotrufo 2016). While microbial immobilisation is restricted to the organic layer and the uppermost mineral layer, the amount of available adsorption sites is generally increasing with soil depth. The adsorption process is influenced by mineral and protein properties, which affect charge and number of adsorption sites. The presence of antecedent organic matter affects the number of available adsorption sites and lower pH is supposed to foster stronger bond types (Bingham & Cotrufo 2016). Occlusion within an aggregate or spatial separation from microbial decomposers effectively preserves N in soil over long periods of time (von Lützow *et al.* 2006). The size of aggregates can be a key predictor of the relative amount of N retained in SOM (Bingham & Cotrufo 2016). Thus, currently the persistence of organic matter in soil is believed to be an ecosystem property (Schmidt *et al.* 2011), controlled by microbial inhibition, physical protection and/or chemical stabilization (von Lützow *et al.* 2006).

An simple approach of nitrogen accumulation is used in Germany since 1995 to estimate N_i in critical loads calculation (CCE 1995). It is assumed that N_i and mean annual temperature (MAT) are negative correlated. Stuhrmann (2000) incubated 85 intact soil columns (organic layer +20 cm mineral soil) from Germany, Sweden and France at different temperatures (5°C, 10°C and 15°C) in laboratory and under field conditions. The columns were pulse-labelled with tracer ^{15}N ($^{15}NH_4^+$). After 6 month of incubation the N and ^{15}N in different soil fractions (microbial, inorganic and organic N) as well as ^{15}N in soil percolates were investigated. Between 11 and 74 % and between 9 and 29% of applied ^{15}N were found in microbial and non-hydrolysable organic N fraction, respectively. Among the different N storage mechanisms only incorporation of N into non-hydrolysable organic N showed a significant positive temperature dependency. Between microbial immobilisation and temperature none (soil columns of three sites) or a negative (soil columns of one sites) correlation were found.

2.2 N stocks and N accumulation rates

In boreal and temperate forests the soils represent the largest sink of the ecosystem nitrogen (Van Miegroet *et al.* 1992; Stuhrmann 2000; Merilä *et al.* 2014). The current nitrogen pools in soils are the result of soil formation and succession. In an idealized climax forest with a mature soil, the total nitrogen, by definition of a steady state, has reached a constant limiting value (Olson 1958). It is assumed that soil organic matter shows an initial period of rapid increase during the soil formation followed by a lower rate of accumulation that may continue for millennia and reach an approximate steady state (VandenBygaart & Protz 1995; Birkeland 1999). Because N inputs are stored in organic pools, N should accumulate most rapidly when organic matter is accumulating rapidly. Thus the capacity to accumulate nitrogen in soil differ due to the successional stage and the maturity of the ecosystem (Van Miegroet *et al.* 1992).

Soil chronosequences give us an indication of nitrogen dynamics during soil formation (Table 1). Soil N stocks increased rapidly at the early stage of soil development and then reached the peak and levelled off, or increase at a slower rate, or declined with increasing soil age. Thus at initial stage of soil formation N accumulation rates can be very high during first centuries (Dickson & Crocker 1953; Crocker & Dickson 1957; Gerlach *et al.* 1994; Jones *et al.* 2008; Rhoades *et al.* 2008; Turk & Graham 2009). Although soil chronosequences over much longer time periods show a continuously decreasing rate of N accumulation, with average rates of 4 kg ha^{-1} yr^{-1} over 1000 years (Olson 1958; Syers *et al.* 1970) and 1 kg ha^{-1} yr^{-1} over 10 000 years (Syers *et al.* 1970). The magnitude and the timing of N accumulation vary but intermediate and late successional ecosystems always contain more N than the youngest ecosystems (Dickson & Crocker 1953; Lichter 1998) with high N accumulation rates during first decades and centuries and slower rates with increasing maturity of the soil.

To calculate N accumulation rate Grennfelt (1992) recommended to divide the total amount of N in soil with the number of years for soil formation. By using this approach a constant (linear) process of accumulation through soil formation is assumed (Table 2). Johnson and Turner (2014) summarized that in most temperate forest ecosystems N contents are less than 10 000 kg ha^{-1} (vegetation + soil). They reviewed data of Cole and Rapp (1981), where N pool of organic layer and mineral soil is smaller in sites with glacial parent material with an average of 4843 kg ha^{-1}, than in sites with sedimentary parent material with averaged 8845 kg ha^{-1}. The overall average N content is 6896 kg ha^{-1} and the median is 5922 kg ha^{-1}. Nitrogen accumulation was mostly less than 1 kg ha^{-1} yr^{-1} in glacial and mostly less than 0.5 kg ha^{-1} yr^{-1} in non-glaciated forest ecosystems. Egli et al. (2012) found an increase of N stocks with increasing soil age till an asymptotic value of approximately 10 000 kg ha^{-1} in mineral soil (till C-horizon) of the Swiss Alps and Rocky Mountains (USA). The accumulation rate ranged from 0.01-100 kg ha^{-1} yr^{-1} for pioneer plant communities, shrubs, grassland and forest. Very young soils can reach very high accumulation rates, but rates drop below 1 kg ha^{-1} yr^{-1} and 0.1 kg ha^{-1} yr^{-1} after approximately 10.000 and 20.000 years of soil age, respectively. If only forest sites were taken into account the N sequestration rates varied between 0.1 and 0.6 kg ha^{-1} yr^{-1} with soil age between 11.000 and 130.000 years.

However the results from long-term N accumulation rates mentioned above are contradicted by results of input-output budgets and studies of short-term changes in soil N stocks (Table 3). This current N accumulation rates can show positive expression with rates between 1.8 and 42 kg ha^{-1} yr^{-1} (Ulery *et al.* 1995; Brinkmann & Nieder 2002; Meiwes *et al.* 2002; Schulte-Bisping & Beese 2016) as well as negative rates (Kreutzer *et al.* 2009; Andreae *et al.* 2016). Changes in N stocks often vary in different soil depths and can also change from accumulation in one layer to mobilisation in another (Andreae *et al.* 2016).

Table 1: Nitrogen accumulation rates from chronosequence studies in kg N ha^{-1} yr^{-1}. Data is sorted by soil age. N.A. = information are not available

Country, site	Vegetation	MAP (mm yr-1)	MAT (°C)	Soil depth (cm)	N accumulation rate (kg N ha^{-1} yr^{-1})	Soil age (yr)	Chrono-sequence	References
UK, Wales	N.A.	850	11	15	67 – 16	10 140	sand dune	Jones *et al.* (2008)
USA, California	Incense cedar	855	12.3	OL 30	8.0	0 – 244	debris flow	Turk and Graham (2009)
USA, Alaska	Pioneer plants, shrubs, balsam poplar, white spruce	240	-5.7	30	24 – 16	0 – 300	floodplain	Rhoades *et al.* (2008)
USA, Alaska	No vegetation, Willow, Poplar, white spruce, black spruce	270	-3.3	OL 20	37 – 1.2	0 – 250	floodplain	Kaye *et al.* (2003)
Germany, Spiekeroog	No vegetation – European alder	N.A.	N.A.	30	7.1 – 2.2	1 – 250	sand dune	Gerlach *et al.* (1994)
New Zealand	Mixed conifer–broadleaf tem-perate rain forest	3455	11.3	OL 100	18.3 – 0.8	181 – 6500	sand dune	Turner *et al.* (2012)
USA, Lake Michigan	Grasses, shrubs, mixed conifer-ous forest with red pine and white pine	772	6.2	OL 15	3.2	0 – 440	beach-ridge	Lichter (1998)
USA, Lake Michigan	mixed coniferous forest with red pine and white pine	772	6.2	OL 15	±0	440 – 4150	beach-ridge	Lichter (1998)
USA, Lake Michigan	Marram, sand reed, little bluestem grasses, cottonwood, sand cherry, jack or white pine, lack oak	850	10	10	4.03	<1 – 1000	sand dune	Olson (1958)
USA, Lake Michigan	cottonwood, sand cherry, jack or white pine, lack oak	850	10	10	±0	1000 – 8000	sand dune	Olson (1958)

Table 2: Age specific nitrogen accumulation rates (N stocks/soil age) in kg N ha^{-1} yr^{-1}. A constant process of nitrogen accumulation through soil formation is assumed. Data is sorted by soil age. N.A. = information are not available

Country, site	Vegetation	MAP (mm yr-1)	MAT (°C)	Soil depth (cm)	N accumulation rate (kg N ha^{-1} yr^{-1})	Soil age (yr)	References
Switzerland, Alps USA, Rocky Mountains	pioneer plants, shrubs, grassland, forest	1100 –2000 400 –1000	-2 – 7.5 -4 – 3	MS till C horizon	0.01 – 100	10 – 1 200 000	Egli *et al.* (2012)
Switzerland, Alps	european larch, common juniper	1300	1.5	50	0.62	10 840	Egli *et al.* (2012)
Switzerland, Alps	larch, Swiss pine heath	1250	-1.1	70	0.47	14 900	Egli *et al.* (2012)
Switzerland, Alps	european larch, common juniper	1300	1.5	60	0.34	17 300	Egli *et al.* (2012)
USA, Rocky Mountains	limber pine, common juniper	550	1.4	50	0.2	22 000	Egli *et al.* (2012)
USA, Rocky Mountains	limber pine, juniper sagebrush	500	2.6	85	0.13	65 000	Egli *et al.* (2012)
USA, Rocky Mountains	limber pine, common juniper	600	1.2	100	0.1	130 000	Egli *et al.* (2012)
Sweden	N.A.	N.A.	N.A.	B horizon	0.2 – 0.5	8700 – 12 400	Rosen *et al.* (1992)

Table 3: Short-term nitrogen accumulation rates in kg N ha^{-1} yr^{-1} from times series and experiments. N.A. = information are not available

Country, site	Vegetation	MAP (mm yr-1)	MAT (°C)	Soil depth (cm)	N accumulation rate (kg N ha^{-1} yr^{-1})	Method	References
Germany, Neuglobsow	pine, beech	658	7.9	OL 80	1.82	ICP Integrated Monitoring, Budget studies	Schulte-Bisping and Beese (2016)
Germany, Höglwald	spruce	933	7.7	OL 10	-8	Budget studies	Kreutzer *et al.* (2009)
Germany, NFSI	forest	478 – 2095	1.9 – 10.6	OL 60	-8.94	German National Forest Soil Inventory, changes in N stocks between 1987-1993 and 2006-2008 (n=1168)	Andreae *et al.* (2016)
Germany, NFSI	forest	478 – 2095	1.9 – 10.6	OL 30	5.66	German National Forest Soil Inventory, changes in N stocks between 1987-1993 and 2006-2008 (n=1218)	Andreae *et al.* (2016)
USA, California	chaparral	678	14.4	100	9.8 – 29.3	Lysimeter experiment	Ulery *et al.* (1995)
Germany, Solling	Beech, spruce	1068	6,4	OL	21 42	Changes in N stocks between 1968 and 2001	Meiwes *et al.* (2002)
Germany, Lower Saxony	pine	N.A.	N.A.	OL	25 – 35		Brinkmann and Nieder (2002)

3 Materials and methods

3.1 Site description and element analyses

Data of soil profiles at forest sites in France, Switzerland and Germany were evaluated in order to see how high stocks are in different countries based on inventory data.

We used soil data from 310 Swiss plots where soil sampling was executed between 1984 and 2011 (FOEN 2017). Organic layer and mineral soil were sampled in diagnostic horizons till a maximum soil depth of 345 cm (mean of 115 cm). Total nitrogen was quantified by Kjeldahl digestion and colorimetric determination of ammonium.

The French soil data of 104 plots from RENECOFOR monitoring network (French part of ICP Forests level II) were sampled in two field campaigns, first between 1992 and 1995 and second between 2007 and 2012. For both campaigns on each site 25 soil pits till 40cm depth were dug on 5 subplots. For each subplot a composite sample were made. Mineral layer from 40 cm to 1 m depth were sampled once in 1998. Total nitrogen was measured by Kjeldahl digestion (mineral soil 10-20 cm and 20-40 cm) and by gas-chromatographic elemental analysis (other depths).

For Germany soil data from the National Forest Soil Inventory (NFSI) were used. First NFSI was carried out from 1987-1993 on about 1900 forest plots, the second inventory followed – about 15 years later - between 2006 and 2008 on about 1800 plots. Organic layer and the upper mineral soil were sampled on 8 satellites as composite samples. Sampling of the mineral soil was implemented at the depth increments of 0–5, 5–10 and 10–30 cm. For lower soil depths composite samples of 3 subsamples from satellites or soil pit were made till 90 cm depth. In this paper we used the data from second German NSFI because of consistent methods of determining nitrogen concentrations between the federal states. Total nitrogen concentrations of all samples (OL and mineral soil) were determined in sieved (<2 mm) samples using gas-chromatographic elemental analysis.

A comparison between nitrogen concentrations analysed by Kjeldahl digestion and gas-chromatographic elemental analysis result in no systematic differences between the methods (Russ *et al.* 2011). Thus all laboratory analysis methods were included in the subsequent analysis.

Nitrogen stocks were used as a proxy to calculated N accumulation rates but there are subjected by anthropogenic influences (liming, depositions) of course. All French and Swiss forest plots were not limed. In Germany 495 of forest plots were limed until the second inventory was carried out. Limed plots were included in subsequent analysis.

3.2 Statistical analyses

Statistical analyses were performed with SAS 9.4 (SAS Institute Inc., Cary, NC, USA). The soil data of all countries was converted in the following soil depths: organic layer, 0-5, 5-10, 10-20, 20-40, 40-60 and 60-80 cm. N pool (kg ha^{-1}) was calculated by multiply fine earth stock with N concentration. If N concentration was lower than the limit of quantification (LOQ) neither N stock was calculated. The N stocks of Germany were weighted by the ratio of forest area of federal state to Germany's total forest area. For overall statistic the N stocks of each country were weighted by their amount of the total forest area. The forest area was 1.21 Mio.[1] , 10.32 Mio.[2] and 16.42 Mio.[3] hectares for Switzerland, Germany and France, respectively.

1 http://www.lfi.ch/publikationen/publ/lfi3.php, forest without shrubs

2 https://www.thuenen.de/media/publikationen/thuenen-report/Thuenen_Report_43.pdf, page 8

The long-term accumulation rate is obtained by dividing the total N pools with the number of years for soil formation. For rough estimation of soil age we used the extension of the last glacial maximum (LGM) to classify the sites into glacial and periglacial plots by an ArcGIS analysis (ESRI 2016, ArcGIS Desktop, Release 10.4.1 Redlands, CA, USA, Environmental Systems Research Institute). The method was recommended by Rihm (2017).

Normality of residuals was checked with a Shapiro-Wilk test and visually by using diagnostic plots. N stocks were log-transformed prior to correlation analyses.

4 Results of soil data

4.1 Total nitrogen stocks in organic layer and mineral soil

Nitrogen pools in the organic layer (OL) ranged from 0 to 10 307 with a median of 372 kg ha^{-1}. The OL-pool of nitrogen is highly variable. Stocks of nitrogen in OL were highest in Switzerland, storing 1 666 kg ha^{-1}, as compared with 671 kg ha^{-1} in Germany and with 258 and 264 kg ha^{-1} in France during first and second field campaign, respectively (Table 4). Nitrogen in the OL represented from 5 %, 12 % to 18 % of the total soil N pool in France, Germany and Switzerland respectively. Mineral soil stocks (0-60cm) ranged from 224 to 46 100 with a median of 4 555 kg ha^{-1}. The mineral N-pool was highest in Switzerland with 7 092 kg ha^{-1}, as compared with 4 643 kg ha^{-1} and 4 324 kg ha^{-1} kg in Germany and France respectively. Total soil pools (OL-60cm) ranged from 850 to 37 455 with a median of 5 215 kg ha^{-1}. The total soil pool of nitrogen was highest in Switzerland, storing 8 042 kg ha^{-1}, as compared with 5 421 kg ha^{-1} and 5 047 kg ha^{-1} kg in Germany and France respectively. N stocks and concentrations were highly variable. There is significant spatial variability in N stocks in organic layer between all countries. Also significant differences in total N soil stocks were found among countries (ANOVA, log-transformed, $p<0.05$). Differences occurred between Switzerland and Germany as well as between Switzerland and France (Tukey t-test).

Despite of the high variability of total N stocks, the vertical distribution of N in the profile is quite similar between the countries (Figure 1). On average, more than 90 % of total N in soils was stored within the mineral horizons. Organic layer and the uppermost mineral soil (OL-10cm) represented 37 % of the total soil N pool (OL+0-80cm). Nitrogen in the OL represented approximately 7 % of the total soil N pool.

3 http://inventaire-forestier.ign.fr/IGD_2015_EN/EN_CRITERE_1.pdf

Figure 2: Distribution of total N in soil profile. Percentage of N stocks (median) in organic layer and mineral soil increments on total soil N pool (OL-80cm).

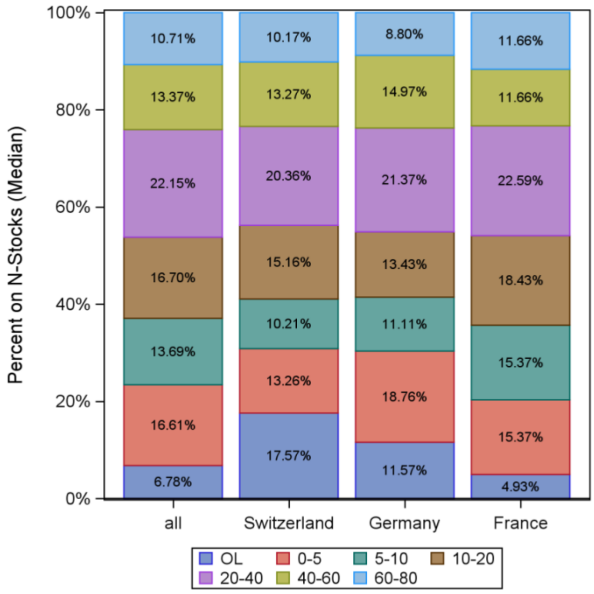

Table 4: Total nitrogen stocks [kg ha^{-1}] in organic layer (OL) and mineral soil increments of France, Germany and Switzerland. N-Pools for mineral soil till 40cm (MS40) and 60cm (MS60) and N-pools of total soil (organic layer + mineral soil) till 40cm (TS40) and 60cm (TS60).

Depth	France 1992-1998				France 2007-2012				Germany 2006-2008				Switzerland 1984-2011			
	P25	Median	P75	n	P25	Median	P75	n	P25	Median	P75	n	P25	Median	P75	n
OL	107	258	571	102	118	264	514	101	239	671	1219	1798	795	1666	3003	63
0-5	608	806	1098	101	669	853	1102	101	756	1088	1426	1846	873	1258	1672	308
5-10	608	806	1098	101	669	853	1102	101	421	644	962	1846	629	969	1347	310
10-20	681	967	1420	100	562	839	1299	99	489	779	1186	1832	926	1438	2072	303
20-40	839	1185	1975	100	654	947	1648	99	771	1240	1863	1841	1277	1930	2678	304
40-60	356	612	949	82					468	868	1321	1811	818	1258	1939	285
60-80	356	612	949	82					284	511	919	1617	536	964	1470	255
MS40	2822	3751	5232	99	2639	3557	4794	98	2623	3743	5394	1827	4100	5819	7578	297
MS60	3352	4324	6263	82					3166	4643	6707	1797	5106	7092	9578	278
TS40	3154	4558	5687	99	3134	4122	5245	98	3400	4562	6135	1771	5207	7127	10763	57
TS60	3647	5047	6506	82					3966	5421	7387	1744	6082	8042	12720	56

4.2 Nitrogen accumulation rates

Glaciated sites with presumably 10.000 years of new N input represent 72 %, 15 % and 7 % of the data of Switzerland, Germany and France, respectively. N accumulation rates of glacial sites (n=265, OL-60 cm) ranged between 0.18 and 3.1 kg ha^{-1} yr^{-1}. The median of N accumulation rates of glacial sites are 0.4 kg ha^{-1} yr^{-1} for German and 0.8 kg ha^{-1} yr^{-1} for Swiss and French sites. Assuming that periglacial sites are at least 24 000 years old the N accumulation ranged from 0.035 to 1.6 kg ha^{-1} yr^{-1} (n=1617) with a median of 0.2 kg ha^{-1} yr^{-1} for France and Germany and 0.4 kg ha^{-1} yr^{-1} for Switzerland. This calculation is very rough and is an overestimate in cases where soils were much older than 24 000 years BP. Nevertheless, this calculation suggests that N immobilisation in OL and mineral soil till 60 cm soil depth is less than 1.5 kg ha^{-1} yr^{-1} (90 percentile).

Table 5: Nitrogen accumulation rates [kg ha^{-1} yr^{-1}] calculated by dividing N stocks by estimated soil age. Sites were classified into glacial and periglacial plots by considering the extension of the last glacial maximum (LGM). The presumed soil ages are 24 000 and 10 000 years for periglacial and glacial sites, respectively. Soil age of Swiss plots were estimated by Rihm (2017). Liming effect in Germany was considered. Liming 0=no liming; liming 1= limed plots

Country	Soil age (Jandl *et al.*)	Liming	P25	Median	P75	P90	Max	N
France	24 000	0	0.2	0.2	0.3	0.4	0.5	79
Germany	24 000	0	0.2	0.2	0.3	0.4	1.6	1057
Germany	24 000	1	0.2	0.2	0.3	0.4	0.8	470
Switzerland	24 000	0	0.3	0.4	0.4	0.4	0.6	11
France	10 000	0	0.4	0.8	1	1	1	3
Germany	10 000	0	0.3	0.4	0.7	1	3	205
Germany	10 000	1	0.4	0.6	0.7	0.8	1	12
Switzerland	6 700 – 12 000	0	0.5	0.8	1.3	1.5	3.1	45

4.3 N_i effecting parameters

The results of the correlation analyses are represent in Table 6. We found significant negative correlation between N stocks and mean air temperature in all soil compartments with correlation coefficients between -0.1 and -0.2 in mineral soil and total soil, respectively. Figure 2 (Appendix) shows N stocks and temperature classes in total soil till 40cm for each country. In contrast N stocks are significant positive correlated with mean annual precipitation (MAP) with a correlation coefficient between 0.2 and 0.35 in MS and TS. N stocks of organic layer were significant negative correlated to MAP with a small correlation coefficient of -0.08. In all compartments the altitude and the N stocks are significant positive correlated with correlation coefficients between 0.009 and 0.25 (not significant in TS 60cm). The strongest significant correlation with coefficients between 0.76 and 0.98 was found between N stocks and C stocks.

Table 6: Pearson's correlation coefficient for relationships between site properties (MAT=mean annual temperature; MAP=mean annual precipitation; Altitude and C stocks) and N pools in different soil compartments (OL=organic layer; MS40=0-40cm, MS60=0-60cm, TS40=total soil OL+0-40cm, TS60=total soil OL+0-60cm), level of significance (** p<0.0001; * p<0.0005).

layer	MAT	MAP	Altitude	C Stock
OL	-0.19**	-0.08**	0.009**	0.988**
MS40	-0.114**	0.329**	0.234**	0.843**
MS60	-0.11**	0.346**	0.251**	0.825**
TS40	-0.218**	0.238**	0.227**	0.78**
TS60	-0.206**	0.27*	0.255	0.762**

5 Discussion and Suggestions

Due to the short lifetime of microorganism biotic N immobilisation is not even a durable storage of N in case of critical loads definition. Microbial immobilisation is restricted to the organic layer and the uppermost mineral layer. The processes which cause long-term accumulation in soil – adsorption to mineral surfaces and spatial separation – taking place in lower soil depths due to increasing of adsorption sites. Already Rosen *et al.* (1992) mentioned that the built up of stable C-N-compounds mainly appear in the B-horizon of soil profile. Furthermore mineral soil represents the major part of the total soil N stock. With regard to international monitoring programs as a data source for critical loads calculation it can be useful to calculated N immobilisation till 40 cm soil depth. In international monitoring programs soil sampling are common till 40 cm. With increasing soil depth there is an increasing in N values below the limit of quantification. In 60-80 cm soil depth 14 % of German and Swiss nitrogen concentrations are below LOQ. Overall 76 % of total nitrogen is located in organic layer and mineral soil till 40 cm depth.

Dividing N stocks by soil age is assumed to be an appropriate approach to calculation N_i. Because the long-term N accumulation is assumed to be very slow (serval hundred years) and it is taken to be about 1 to 3 kg ha^{-1} yr^{-1} (Nilsson & Grennfelt 1988). Our results are consistent with Rosen *et al.* (1992) and Johnson and Turner (2014) who estimated the annual N immobilisation since the last glaciation at between 0.2–0.5 kg ha^{-1} yr^{-1} and 0.5–1.0 kg ha^{-1} yr^{-1}, respectively. The age specific nitrogen accumulation rates represent the maximum potential for present accretion of nitrogen in soils, because a constant (linear) process of accumulation through soil formation is assumed. The accumulation rates of young soils and peat soils can be different. On the other hand current changes in N stocks by time series are a present-day snap shot (even if observations span over several decades) which are very likely influenced by anthropogenic processes (e.g. high N-deposition, management) or natural short-term disturbances (e.g. storm events, wildland fire or bark beetle calamities).

Current N pools in Germany and Switzerland are the result of soil development in the Holocene. But especially for countries without Pleistocene glaciation the soil age can be different. Thus for an European approach to estimate soil age and thus N immobilisation it is necessary to link the N stocks (e.g. derived from international monitoring programs) to the distribution of dominant soil types/parent material according to the Soil Map of Europe (JRC). The distinction between soil types includes parameter like clay content and C/N ratio which are supposed to be important for long-term N accumulation.

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8 Appendix

Figure 3: N stocks (t ha-1) in total soil till 40cm depth and mean annual temperature (MAT) classes at all sites (left top), at Swiss sites (right top) at German sites (left bottom), and at French sites (right bottom). Please note only 57 Swiss plots have complete N stocks for organic layer + mineral soil till 40cm.