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Soil nitrogen explanatory factors across a range of forest ecosystems and climatic conditions in Italy



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ABSTRACT

N is known to be the most limiting element for vegetation growth in temperate and boreal forests. The expected increases in global temperature are predicted to accelerate N mineralization, therefore incrementing N availability in the soil and affecting the soil C cycle as well. While there is an abundance of C data collected to fulfill the requirements for national GHG accounting, more limited information is available for soil N accumulation and storage in relation to forest categories and altitudinal gradients. The data collected by the second Italian National Forest Inventory, spanning a wide range of temperature and precipitation values (10° latitudinal range), represented a unique opportunity to calculate N content and C/N ratio of the different soil layers to a depth of 30 cm. Boosted Regression Tree (BRT) models were applied to investigate the main determinants of soil N distribution and C/N ratio. Forest category was shown to be the main explanatory factor of soil N variability in seven out of eight models, both for forest floor and mineral soil layers. Moreover latitude explained a larger share of variability than single climate variables. BRT models explained, on average, the 49% of the data variability, with the remaining fraction likely due to soil-related variables that were unaccounted for. Accurate estimations of N pools and their determinants in a climate change perspective are consequently required to predict the potential impact of their degradation on forest soil N pools.

1. Introduction

The global carbon (C) cycle is increasingly affected by elevated nitrogen (N) deposition due to increased fossil fuel combustion and agricultural practices. Soil contains the largest pool of fixed and biologically available N (Bingham and Cotrufo, 2015), therefore, understanding the impacts of N cycling on C, water and energy exchanges in terrestrial ecosystems is necessary to produce accurate climate change scenarios that account for the major feedback mechanisms, in particular those related to plant and soil nutrient status (Huang et al., 2016). In addition, N cycling in forests is projected to be most affected by future global warming (Bai et al., 2013). N is present in soil in both organic (mainly as amides, amines and aromatic N compounds) and inorganic (mainly as ammonium and nitrates but also in micas-containing soils, see Dahlgren (1994)) forms (Bingham and Cotrufo, 2015) but most of

the N in forest soils is stored in organic compounds such as proteins, lignin or chitin (Rennenberg and Dannenmann, 2015). Soil C/N ratios are recognized as good indicators of ecosystem N status, nitrate leaching to ground and surface waters (Dise et al., 1998; Gundersen et al., 1998) and indicators of N-availability (Brady and Weil, 1998). Since C and N accumulate simultaneously in organic matter, the long-term accumulation of C per unit N in a compartment cannot exceed a certain range in C/N stoichiometry of that compartment and, as a consequence of different C/N ratios, much more N is required to sequester C in soils, foliage and roots than in woody biomass (de Vries et al., 2009). According to Cools et al. (2014), a combination of variables should be investigated in order to identify the main determinants of soil C/N ratio at the European scale: among those, the most important factors are tree species, ecological region, soils and humus type. Moreover, in order to determine the C sequestration potential of soils, it

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is imperative that soil N stocks and C/N ratios are quantified (Finzi et al., 1998; Luo et al., 2006; de Vries et al., 2009).

The productivity of many temperate forests is N limited (de Vries et al., 2009), therefore N input via deposition, has the potential to increase growth and sequester CO_2 from the atmosphere. European forests receive N inputs as wet and dry depositions, in the range of $1-20 \text{ kg N ha}^{-1} \text{ y}^{-1}$ both for NH₄-N and NO₃-N, with the majority of high deposition sites situated in central Europe (Michel et al., 2015). With increasing N-enrichment, N immobilization will decrease (N leaching will increase), C/N will decline and consequently less C will be sequestered per unit N deposition.

Despite a great abundance of ecosystem scale carbon related information, limited data are available for soil N accumulation and storage in natural ecosystems, in relation to forest category or along altitudinal gradients (Bingham and Cotrufo, 2015; Tashi et al., 2016). The second Italian National Forest Inventory (Gasparini and Tabacchi, 2011), conducted between 2002 and 2006, had the objective of collecting the carbon-related information required for the Kyoto Protocol reporting. With an average stock of 81.7 Mg C ha $^{-1} \pm 1.4$ (Standard Error of the Mean), soil was reported to be the main C pool, including more than 50% of the total forest organic C (Gasparini and Di Cosmo, 2015).

The main aim of the present study was to investigate the explanatory factors of the N content and C/N ratio of Italian forest soils by means of BRT models in order to improve our knowledge regarding soil N predictors at a regional scale, spanning a wide range of forest ecosystems and climatic conditions.

2. Materials and methods

2.1. Data availability

A complementary phase of the Italian NFI, was carried out during the 2008 and 2009 growing seasons on a subsample of 1499s-phase NFI sample plots, stratified by administrative region and forest category, aiming at determining the C stock of forest floor and soils, understory regeneration, shrubs and deadwood as well as living trees (Gasparini and Di Cosmo, 2015). Aboveground biomass and soil data were collected on circular plots of 13 m radius. Inside each plot, three sub-plots for litter and soil sampling were systematically located at 12 m from the plot centre in three different directions (N, SE and SW). If obstacles were present (e.g. boulders, trees, stumps), the sub-plot was moved by 0.5 m increments along the same transect, towards the centre of the plot, until a new, suitable, obstacle-free point was found (detailed information about the sampling design are available in the NFI protocols; Gasparini and Tabacchi, 2011). A final number of 1404 plots were used for data analysis, 95 plots were rejected due to data unavailability (e.g. high presence of boulders, other obstacles or because they ended up on impervious terrain). Only the raw N data (determined by FEM using the dry combustion technique) were used for the present study to calculate the soil N stocks, the results we got might therefore slightly diverge from the official NFI statistics due to different data elaboration methods.

2.2. Soil sampling and N determination

Detailed information on the field sampling protocol and the adopted definitions for soil layers are available in: Gasparini and Tabacchi (2011), Gasparini et al. (2013) and Gasparini and Di Cosmo (2015), therefore only a brief overview is provided here. On each of the three sub-plots, litter (L) and organic layers (FH = fermentation-humus layers) were collected within a 900 cm 2 square surface delimited by a 30×30 cm aluminum frame. The boundary between the FH and mineral layers was considered as the "0 level" for soil sampling. In order to estimate the stone and root content of soil, the excavation method (Page-Dumroese et al., 1999) was preferred to the corer sampling

method (Rodeghiero et al., 2010). The mineral soil was excavated down to 30 cm from a 20×20 cm square surface, systematically located inside the litter sampling frame and divided into: surface mineral soil layer (from the "0 level" to 10 cm depth) and sub-surface mineral soil layer (from 10 to 30 cm). For each soil layer, a homogeneous subsample (for a detailed description of the method see Rodeghiero et al., 2010) of the excavated material was carried to the lab, dried, weighted and separated into its components (soil, stones, roots). The remaining "nonhomogeneous" part (big stones and roots) was measured in the field and left on site. The exact depth of each excavated layer was measured in four points and recorded (the theoretical depth may not have been reached due to the presence of stones and/or roots). Mineral soil bulk density (Bd; g cm⁻³) was determined by sampling known volumes of soil along the perimeter of the pit (Rodeghiero et al., 2009), using 100 cm³ metal cylinders (Eijkelkamp, Giesbeek, the Netherlands). The main soil components (fine earth, stones and roots) were separated by sieving (at 2 mm) in the lab and weighed. The total C and N content of L, FH and mineral soil samples was measured with a PerkinElmer (Norwalk, CT, USA) PE2400 CHNS/O elemental analyzer.

The N stocks reported in this study do not include woody components (twigs, branches, bark, roots) because the N content of these pools was not available. The N stock (g N m $^{-2}$) of the L and FH layers (i.e. the forest floor) was calculated as dry mass multiplied by the N concentration (N%) divided by the slope-corrected pit area (A $_{LFH}=30\times30$ cm) projection:

$$N_{LFH} = \frac{\text{dw N\% 100}}{A_{LFH}\cos(\alpha)} \tag{1}$$

where N_{LFH} is the N stock (g N m⁻²), dw is dry weight of the considered layer (g m⁻²) and α is the slope in degrees.

In case of incomplete excavation of a mineral layer (according to the depth reported in the sampling protocol), the layer fraction was adjusted to 10 or 20 cm: *i.e.* an excel worksheet was used to automatically retrieve the missing amount of soil from the underlying layer, if present, whereas excess soil (*i.e.* excavated depth > 30) was discarded. This depth adjustment was adopted to have in all plots a comparable sampling depth. In presence of stones and boulders larger than the pit surface (20×20 cm), the underlying volume was considered as bedrock for the remaining layer, to a depth of 30 cm (Rodeghiero et al., 2010) whereas smaller rocks were sampled together with the soil. The N stock of mineral soil layers (N_{M_i} ; g N m $^{-2}$) was calculated as:

$$N_{M} = \frac{N\%Bd V H_{F}}{A_{M}cos(\alpha)}$$
 (2)

where Bd is the fine soil bulk density (g m⁻³); V is the reference soil volume (i.e. a soil layer with defined thickness and 1 m² surface); H_F is a dimensionless factor, which represents the fine soil fraction content in the soil volume V, calculated as (1 – (stone volume + root volume)/V) (adapted from Rodeghiero et al., 2009); A_M is the sampling surface of the mineral soil (20 \times 20 cm); α is the slope in degrees. The total N content of each excavated pit, to a depth of 30 cm, was calculated as the sum of the single layers. The total profile C/N was calculated as the ratio between the total C and the total N of the excavated profile. The mean C/N ratio of the FH layer was calculated for the 1404 plots and divided into three categories: C/N < 15; $15 \le C/N \le 25$; C/N > 25with the purpose to check it's variability at regional level. The cutoffs (15 and 25) were chosen based on the findings of MacDonald et al. (2002) and Gundersen et al. (2009) about nitrate leaching and given the correspondence of C/N ratio with the soil organic matter decomposition and humus type (Mull C/N < 15; Moder C/N \approx 15–25; Mor C/N > 25; Duchaufour (1983), Brethes et al. (1995), Gobat et al. (1995)). The mean plot (or mean layer) N content was then obtained as the average of the three pits (or layers). A quality check procedure was conceived for the mineral soil Bd values, since the sample collection was quite meticulous, time demanding and subject to large errors especially in stony soils. Missing or unusual Bd values (i.e. outside the

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