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# Atmospheric ammonia concentration modulates soil enzyme and microbial activity in an oak forest affecting soil microbial biomass

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ABSTRACT

The present work was carried out to assess the effect of atmospheric ammonia ($NH_3$) on soil physicochemical properties, soil enzymatic activities (β-glucosidase -β-GLU-, nitrate reductase –NR-, urease –UR-, protease –PRO-, acid phosphatase –PHO-, dehydrogenase –DHA-), soil microbial biomass and soil respiration. The study was conducted along a $NH_3$ gradient in a *Q. pubescens* Milld. forest in the vicinity of two livestock farms. Because of $NH_y$ ($NH_y$: $NH_3$ and $NH_4^+$) deposition, N saturation was detected up to 330 m from the farms. This excess of N led to a decrease in soil C:N and an increase in soil nitrification processes, which resulted in an accumulation of the heavy N isotope ($^{15}N$) in the soil. N saturation was also reflected in the activity of NR enzyme, which was inhibited. On the other hand, while UR enzyme was inhibited close to the farms possibly due to the high amount of $N\text{-}NH_4^+$ resulting from the hydrolysis of $NH_3$, PRO activity was stimulated by the presence of organic nitrogen compounds and the need of soil organisms to meet the C demand. In addition, the activity of PHO and β-GLU enzymes was regulated by the relative amount of C and P that organisms need. Regarding biological variables, enhanced $NH_3$ reduced soil microbial biomass and biomass respiratory efficiency. Finally, soil enzyme activities and soil microbial biomass have proved to be good biological indicators of soil quality.

## 1. Introduction

Agricultural activities accounted for 93.3% of European ammonia ($NH_3$) emissions in 2013, with animal production and volatilization from livestock excreta responsible for 63% of agricultural $NH_3$ emissions, whereas agricultural soils accounted for the rest (Eurostat, 2015). Inventories from China (Zhou et al., 2015) and North America (Bittman and Mikkelsen, 2009) also found that livestock was the dominant contributing source of $NH_3$ emission, proving this is a global scale problem. Predictions for future scenarios include an increase in $NH_3$ emissions for the coming decades, mainly related to the expected growth of livestock production to satisfy an increasing demand for food and meat consumption per capita, and to changes in climate due to global warming (Fowler et al., 2015; van Vuuren et al., 2011).

Regarding livestock production, about 30–40% of its $NH_3$ emissions arise from livestock buildings (Amon et al., 2016). In this sense, livestock buildings are recognized reactive nitrogen (Nr: includes all the N forms except $N_2$) emission hot spots in its reduced form ($NH_y$: $NH_3$ and $NH_4^+$), since the $NH_3$ emitted is either converted into ammonium aerosol and washed out by rain, or dry deposited. Most of the $NH_3$ is deposited within a few kilometres from the source due to its short atmospheric lifetime. Thus, problems arise when there are sensitive ecosystems as forests, near intensive livestock farms (Loubet et al., 2009; Theobald et al., 2001), since atmospheric nitrogen (N) deposition is well known to cause negative effects on ecosystems.

In soils, N deposition causes acidification that might affect mineralization processes and nutrient availability (de Vries et al., 1995, 2000; Erisman and de Vries, 2000), eutrophication, base cation depletion, increased aluminium toxicity, nitrate leaching (Horswill et al., 2008), changes in N and C cycle and in C:N ratio (Corre et al., 2007; Gundersen et al., 2006; Vitousek et al., 1997), a decrease in soil respiration (Olsson et al., 2005) and changes in the structure of the microbial community (Nemergut et al., 2008).

Soil microbial biomass and enzymatic activities are apparently good indicators of soil quality since they are very sensitive to both natural and anthropogenic factors (Dick, 1997; Utobo and Tewari, 2015). Consequently, they may be useful as early-warning indicators of biological changes in soils (Bandick and Dick, 1999; Masciandaro et al.,

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2004). Moreover, Visser and Parkinson (1992) suggested that the most appropriate soil properties to evaluate soil quality were those related to the recycling of nutrients because they give information about the soil functional state. Therefore, enzymatic activities involved in N, P and C cycles are suitable indicators as they provide information about the soil microbiological status and soil physicochemical properties (Aon and Colaneri, 2001). In addition, soil respiration and metabolic quotient ($qCO_2$) have been reported to elucidate effects of environmental changes on microbial communities (Anderson and Gray, 1991). There is also growing evidence that soil biological properties are affected by environmental factors and may be potential indicators of ecological stress (Bastida et al., 2006; Pascual et al., 2007).

Although some researchers have studied the influence of N fertilization on enzymatic activities and soil respiration in forest ecosystems (Allison et al., 2008; Carreiro et al., 2000; Chen et al., 2002; Compton et al., 2004; Cusack et al., 2011; Frey et al., 2004; Keeler et al., 2009; Saiya-Cork et al., 2002; Thirukkumaran and Parkinson, 2000), little attention has been paid to changes in biochemical and biological variables in soils influenced by atmospheric $NH_3$ concentration, a toxic gas that can diffuse through biological membranes (Andrade and Einsle, 2007).

The aim of this study was to evaluate the effect of atmospheric $NH_3$ on soil physicochemical properties and soil microbial and enzyme activities on a forest ecosystem. Research was conducted in the vicinity of two livestock farms, one pig and the other cattle, which have been running for almost 50 and 10 years, respectively. To that end, we measured soil enzymatic activities, microbial biomass and soil respiration. The enzymes chosen were based on their relevance in the cycling of C (β-glucosidase, β-GLU), N (nitrate reductase, NR, urease, UR and protease, PRO), and P (acid phosphatase, PHO).

The observed changes derived from this continuous source of $NH_3$ should reflect the integrated effect of this pollutant at the ecosystem level. Therefore, unlike N-addition and simulated fertilization studies, which might not be performed long enough, the current study aimed to evaluate the influence of atmospheric $NH_3$ on soil biochemical and biological properties in a forest adapted to high N loads.

## 2. Material and methods

### 2.1. Area of study

The area of study is located in an Oakwood dominated by Q. pubescens Milld.in the north of Spain (42° 55′ 15″ N, 1° 50′ 45″ W). This location is embedded in the transitional region between the Mediterranean and the Oceanic climates, with mean annual precipitation of 1100 mm and mean temperature of 12.5 °C (30 year historical data series, meteo.navarra.es).

At the upwind edge of this woodland, there are two high-intensity point-sources of $NH_3$, two livestock farms (pig and cattle) permanently housing ca. 3600 sows and 950 cows founded in 1966 and 2005, respectively. The area is surrounded by agricultural fields and there is a main road one kilometre from the farms. Management of the site consisted in cattle grazing. As a result of this management, the structure of the forest presented a scarce shrub layer and a dense herb layer. *Rubus* sp. and *Crataegus laevigata* (Poir.) DC. were the most frequent species in the shrub layer whereas *Agrostis capillaris* L., *Geum urbanum* L., *Prunella vulgaris* L. and *Rumex conglomeratus* Murray dominated the herb layer of the forest.

In total, 9 sites were sampled at increasing distances (shown in Table 1) from the livestock farms, ranging from 30 to 1000 m (background point) (Fig. 1).

### 2.2. Air pollution monitoring

Atmospheric $NH_3$ concentration was monitored at the nine sites in 2-week-long periods between July 2013 and July 2015. Triplicates of ALPHA diffusive samplers were used for $NH_3$ measurements (Tang et al., 2001). Radiello® samplers (http://www.radiello.com) were periodically used in order to verify the accuracy of ALPHA devices and travel blanks were used in every sampling ocassion (Puchalski et al., 2011). All samplers were exposed at 1.5 m height.

### 2.3. Soil physicochemical variables

The study was carried out in a clay loam soil (35% clay, 40% silt loam, 25% sand), classified as *Eutric Cambisol* (IUSS Working Group WRB, 2015) whose basic properties (texture, structure, CEC, $CO_3$) were quite homogeneous along the $NH_3$ gradient. Soil samples (0–5 cm) were collected in the nine points as follows: three composite samples (the 3 replicates) made by three subsamples were collected in every sampling point on 1st June 2016. Sample collection was conducted in spring since in Mediterranean ecosystems enzymes display their highest activity in this season, when plants and microbes are in their most active growth (Garcia et al., 1997, 2002).

In the laboratory, the samples were separated for physicochemical and biochemical analyses. Samples for physicochemical analysis were air dried and passed through a 2 mm sieve and ground. Soil pH (ratio 1:2.5 soil:water) was measured and available phosphorus (P) in the soil was determined following the method developed by Olsen et al. (1954), an alkaline extraction of *ortho*-phosphate with sodium bicarbonate followed by a colorimetric determination (630 nm) after reaction of the extract with ammonium molybdate in acidic medium. Soil C and N contents, as well as $\delta^{15}N$, were measured following the methodology developed by Delgado et al. (2013), C and N contents in soils as well as $\delta^{13}C$ and $\delta^{15}N$ signatures, were measured using an Isoprime 100 precision stable isotope ratio mass spectrometer coupled to an Elementar analyzer (MicroCube). $\delta^{15}N$ represents the relative difference, expressed per mil (‰), between the isotopic composition of the sample and that of a standard. The standard used was atmospheric $N_2$:

$$\delta^{15}N\ (‰\ vs.\ atmospheric\text{-}air) = [(R_{sample}/R_{standard})–1] \times 1000$$

where $R_{sample}$ and $R_{standard}$ are the isotope ratio $^{15}N/^{14}N$ for the sample and the standard respectively.

Soil extractable $N\text{-}NO_3^-$ was analysed by ion chromatography after extraction with deionized water (1:2.5 soil:water).

### 2.4. Enzymatic activities

All enzymes assays were conducted with sieved soil (2 mm) at field moisture within one week after sampling. Soil subsamples were oven dried at 105 °C for 48 h to calculate enzymatic activities on a dry weight basis (DW). Before the analysis, linearity tests were performed to adjust the reaction times for every enzyme. For all enzymatic assays, control samples were analysed following the same procedure with exception of the substrate addition, which was conducted after the incubation period and immediately prior to stop the reaction.

Nitrate reductase activity was determined as the amount of $NO_2$ released from 5 g of soil after incubation for 24 h at 25 °C with the substrate 25 mM $KNO_3$ and 0.9 mM 2,4-dinitrophenol solution (Kandeler, 1995). Values of NR activity were expressed as μg $N\text{-}NO_2^-$ g soil $DW^{-1}$ $day^{-1}$ and three replicates per soil sample were measured. UR activity was determined as the amount of $NH_4^+$ released from 0.5 g of soil after incubation for 2 h with the substrate urea (6.4%) at 37 °C in 2 mL of borate buffer (0.1 M, pH 10) (Kandeler and Gerber, 1988). PRO activity was determined as the amount of $NH_4^+$ released from 0.5 g of soil after incubation for 75 min at 40 °C with the substrate 0.03 M N-α-benzoyl-L-argininamide (BAA) in 2 mL of phosphate buffer (0.1 M, pH 7) (Nannipieri et al., 1980). The results of UR and PRO activities were expressed as μg $N\text{-}NH_4^+$ g soil $DW^{-1}$ $h^{-1}$. Phosphatase activity (PHO) was determined as the amount of *p*-nitrophenol liberated from 0.5 g of soil after incubation for 50 min at 37 °C in a shaking water bath using