Nitrogen deposition impact on terrestrial ecosystems

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FLORINA BOTEZ¹, CARMEN POSTOLACHE¹,*
¹Department of Systems Ecology, Faculty of Biology, University of Bucharest, Splaiul Independenței no. 91-95, District 5, 050095, Bucharest, Romania
*Corresponding author; email: carmen_postolache83@yahoo.com

Abstract

Biodiversity loss, as a result of human activities, affects ecosystem productivity and sustainability. Along with climate change and habitat reduction, enhanced nitrogen inputs via atmospheric deposition represent the major drivers of biodiversity reduction. Nitrogen is a limiting factor for ecosystems. Increased bioavailable nitrogen due to increased nitrogen emissions is detrimental for biodiversity compartments. This review summarizes the general soil nitrogen biogeochemical cycle, macro regional nitrogen deposition studies and trends as well as the effects of increased nitrogen deposition, whether site specific or experimental addition. Present review also describes types of impact that elevated nitrogen induce on different ecosystem types and managerial propositions to mitigate the negative impacts of nitrogen depositions.

Keywords: terrestrial nitrogen cycle, microorganisms, biodiversity, grasslands.

1. Introduction

The most important issue of the Natural Capital is the detrimental impact of anthroposphere on ecodiversity. The historical breakthroughs from agriculture and industry have allowed an exceeding access to resources and service provided by the ecosphere (e.g. Vădineanu, 2004). These premises lead to a remarkable development of the global socio-economic system which in turn induced rapid and huge pressures on its foundation comprising natural and semi natural systems. The exponential growth of human population along with its demands for resources resulted in an accelerated erosion of the biosphere (Sutton et al., 2011). It is worldwide accepted that this development surpassed the supporting capacity of the Natural Capital. Moreover, the negative forms of impact may develop at different scales in time and space and can present a synergic potential, which can hinder a sustainable management of the resulting ecological issues (MEA, 2005).

Biodiversity loss, on all its levels from gene to ecosystem, is becoming heightened by the minute and is the main source of the Natural Capital erosion. The principal types of impact on biodiversity (ecodiversity) components resulting from the intensification of anthropospheric metabolism are: natural and semi natural habitat loss, global warming and disruption of biogeochemical cycles of the elements, mainly carbon and nitrogen (e.g. Sutton et al., 2011). Next to carbon, nitrogen is a nutrient of quintessential importance, mostly because the ecosystems productivity is highly dependent on nitrogen availability, which from a historical point of view was mostly very limited (e.g. Vitousek et al., 1997, Eickhout et al., 2006, Spranger et al., 2009). The disruption of nitrogen biogeochemical cycle has the potential to impact all types of ecosystems due to the fact that its bioavailability is crucial for all living organisms (e.g. Wamelink et al., 2007). For most terrestrial or aquatic ecosystems the nitrogen stock is a key regulating factor in controlling the vegetation type and diversity, population dynamics, as well as ecological processes.
Bioavailability and nitrogen inputs are key factors controlling the metabolism of terrestrial ecosystems (Boring et al., 1988, Vitousek et al., 1997, Follett and Follett, 2008). Human activities are responsible for the increased reactive nitrogen flows between biosphere and abiotic compartments: pedosphere, hydrosphere and atmosphere (e.g. International Nitrogen Initiative Steering Committee, 2004). It is estimated that nitrogen emissions are currently more than double compared to the time before agricultural and industrial breakthroughs (Lynch, Kerchner, 2005, Sutton et al., 2009, Raven et al., 2010, Erisman et al. in Sutton et al., 2011). This magnification lead to a considerable nitrogen input for natural and semi natural ecosystems, mainly in the Northern hemisphere. It has been stated that nitrogen deposition represents the third driver of biodiversity erosion, preceded by habitat loss and global warming (e.g. Sutton et al., 2011).

The effects generated by the anthropic nitrogen flows exhibit a complex dynamic in time and space due to atmospheric transport at great distances from point sources of emissions (urban, industrial and agricultural areas) (e.g. Vitousek et al, 1997, Follett and Follett, 2008, Cornell, 2011).

The effects of nitrogen biogeochemical cycle disruption can be summarized as follows:

- Increase of global nitrogen protoxide, potential greenhouse gas, as well as other nitrogen oxides that are responsible for photochemical smog;
- Soil nutrient loss (calcium and potassium) that are essential to preserve soil fertility;
- Significant acidification of soil and aquatic systems;
- Increased nitrogen flows from rivers to estuaries and coastal systems, which has a detrimental impact;
- Acceleration of biodiversity loss, particularly for plant species that are adapted to low levels of available nitrogen, reverberating in the quality and quantity of ecosystems production of resources and services.

2. General aspects of terrestrial nitrogen biogeochemical cycle

The first nitrogen cycle definition was given more than three centuries ago: “It is like a wingless bird that flies day and night without rest; it penetrated between all the elements and carries with it the spirit of life – from nitrum are originated minerals, plants and animals. (It) never perishes; it only changes its form: it enters the bodies of animals in the form of food and then is excreted. It is thus returned to the soil, from which part of it again rises into the air with vapors, and hence it is again among the elements” (Johannes Rudolph Glauber, in Keeney, Hatfield, 2008).

Along with oxygen, carbon and hydrogen, nitrogen is an essential macronutrient for biological systems, in which it is incorporated in chlorophyll, amino acids, nucleic acids, amino sugars and polymers (e.g., Coleman et al., 2004). The average nitrogen content in organisms’ dry weight is 6.25% (Bothe et al., 2007). It can exist in various oxidation states ranging from -3 to +5, depending on the complex stages of oxidation and reduction processes within living organisms. The diversity of biogeochemical transformations along with a reduced bioavailability had a significant impact on plant species at an evolutionary scale and lead to various and energy efficient mechanisms of nitrogen uptake (Boring et al., 1988, van Dobben et al., 2006, Ma et al., 2009).

The most widely represented nitrogen form is molecular nitrogen – approximately 78% of the atmosphere which is the main reservoir of this element, but in this compartment turnover rates
are very slow compared to terrestrial and aquatic systems (e.g. Nieder et al, 2008, Mosier, 2008). Other atmospheric nitrogen forms include nitrogen oxides and ammonia in much smaller amounts. Other global compartments such as oceans and water bodies contain dissolved and particulated nitrogen oxides and ammonia (e.g. Neff et al., 2002). Also, biological systems can be considered a nitrogen reservoir and the most active component of global nitrogen cycle (e.g. Nordin et al, 2009).

Microbial, vascular plants and animal productivity is generally optimal at low levels of bioavailable nitrogen (Vitousek, Howarth, 1991 in Chesworth, 2008, Sutton et al., 2009). In soil, nitrogen is mostly found in the organic soil phase, but also in the root area, microbial biomass and dead organic matter in various stages of decomposition (e.g. Marschner and Rengel, 2007). Regardless of the low levels of reactive nitrogen (ammonium, nitrate and occasionally nitrite) characteristic to most soils, turnover rates are rapid, and seasonal variations of these rates are correlated with microbial activity, directly influenced by weather conditions (e.g. Slinkers et al., 2004, Franzaring and Fangmeier, 2004). Soil also presents various other nitrogen species: dinitrogen, nitrogen oxides, ammonia and other excreta compounds.

Molecular nitrogen is relatively inert from a chemical point of view and it requires costly energy consumption in order to break the triple bond and be biologically assimilated (e.g. Eldor, 2007, Chesworth, 2008). Reactive nitrogen include numerous oxidation states which is a significant trait reflecting upon the various transformations that take place in soils.

Nitrogen cycle comprises both transformations between reduced and oxidized states and transformations between organic and inorganic nitrogen. Organic N takes many forms, such as amino acids (e.g., glycine, glutamine), amino sugars (e.g., glucosamine, galactosamine), nucleosides (e.g., adenine, guanine), peptides, phospholipids (e.g., phosphatidylethanolamine, phosphatidylserine), vitamins (e.g., niacin), and other compounds such as creatine, cyanide, allantoin, various alkylamines, and urea. Unidentifiable forms of N are typically polymerized with soil organic matter fractions such as humic and fulvic acids (Coyne and Frye, 2004).

The main stages of soil nitrogen cycle (Figure 3) can be grouped in five main categories: biological fixation of atmospheric nitrogen, mineralization (ammonification), nitrification, denitrification (nitrate reduction) and assimilation (immobilization). We will detail each step in the following paragraphs.

**Atmospheric nitrogen fixation**

Biological fixation of atmospheric nitrogen is the first and most important stage of nitrogen biogeochemical cycle (e.g. Eldor, 2007, Marschner and Rengel, 2007). This process represents conversion of molecular nitrogen to ammonium by specialized microorganisms (approximately 90 genera of diazotrophic microorganisms that utilize a complex enzyme, nitrogenase). Microbial nitrogen fixation requires substantial energy consumption (eqn [1]).

\[
N_2 + 16\text{Mg}^2+\text{ATP} + 8\text{H}^+ \xrightarrow{\text{Nitrogenase}} 2\text{NH}_2 + \text{H}_2 + 16\text{ADP} + 16\text{Pi} + 16\text{Mg}^{2+}
\]  

Nitrogenase is a complex enzyme consisting of dinitrogenase reductase and dinitrogenase (reduces molecular nitrogen to ammonia) which is highly preserved within microorganisms and its activity is inhibited by oxygen, therefore nitrogen fixation cannot be done in the presence of oxygen (e.g. Nieder et al., 2008). Nitrogenase does not require a specific
substrate, and it can also reduce $\text{H}^+$ to $\text{H}_2$, $\text{N}_2\text{O}$ to $\text{N}_2$, or acetylene to ethane, the latter being of significant importance in identifying and quantifying atmospheric nitrogen reduction in various conditions (e.g. Hillel et al., 2004). Mineral nitrogen is assimilated only by plant species and animal species attain nitrogen (required for the biosynthesis of amino acids) from plants (e.g. Eldor 2007).

The enzymatic reduction of molecular atmospheric nitrogen to ammonia is highly critical for agriculture and ecology because it represents the most significant source of metabolic nitrogen required for living organisms (e.g. Karlovsky, 2008). Soil productivity is strictly correlated with bioavailable nitrogen richness. It is estimated that 100 million tons of dinitrogen are fixated per annum via microbial fixation (approximately 90%, the rest is achieved by lightning and fertilizers (e.g. Nordin et al., 2009).

Boring et al. (1988) have estimated that nitrogen inputs by diazotrophic microorganisms vary between 0 – 30 kg/ha/year, though it has been reported that this capacity can be significantly higher, near 100 kg/ha/year, for desert systems, tropical grasslands or savannah. For grassland ecosystems, the higher values can be explained by favorable conditions such as light, temperature and moist (e.g. Horswill et al., 2008).

Biological fixation is also achieved by symbiotic organisms in which case the nitrogen fixing microorganisms are “rewarded” with carbon (energy source) by plants that assimilate the readily available nitrogen. The supplemental energy in this case offers a better efficiency of nitrogen fixation compared to free-living microorganisms (e.g. Marschner and Rengel, 2007). The adequate amount of mineral nitrogen in soil significantly reduces the rate of biological fixation, thus a dynamic equilibrium is established. Symbiotic nitrogen fixing organisms are frequently present in systems that are limited by nitrogen, either due to low levels of nitrogen or to elevated percolation rates (Boring et al., 1988).

**Mineralization**

The mineralization process is the breakdown of organic matter into inorganic compounds. Organic nitrogen is hydrolyzed and turned into ammonia, which then gains a proton in the soil solution and converted to ammonium ([eqn 2]), therefore this process is also named ammonification.

$$\text{N} - \text{organic} (R - \text{NH}_2) \rightarrow \text{NH}_2 \rightarrow \text{NH}_4^+$$

(2)

On a broader scale this process can be summarized by two distinct phases: proteolysis and actual ammonification.

**Proteolysis (nonspecific phase)**

Complex organic compounds are hydrolyzed to molecules that can enter proteolytic microorganisms’ cells and are further used or degraded in the following stages. The proteolysis process is completed by the functional microbial group of *proteolytic microorganisms*, widely spread at a global scale; this group is heterotroph, without presenting a specific substrate, and are able to synthesize and secrete proteolytic enzymes.

**Proper ammonification (specific phase)**
This specific stage is intermediated by the physiological group of ammonifying microorganisms, and during this process the breakdown of organic nitrogen is completed and ammonia is formed. Ammonification converts organic nitrogen compounds such as nucleic acids, urea, uric acid or amino sugars. The resulting ammonia is mostly given back to the atmosphere, but a fraction of it is temporarily adsorbed on clay-humic composites or converted in the presence of oxygen into ammonium, oxidized to nitrite and nitrate, which is easily absorbed by plants and most microorganisms.

Ammonification rates are higher in well aerated and organic matter enriched soils. Acidity is a regulating factor as well since in this case ammonia production is mainly achieved by fungi. Moreover, carbohydrates enriched soils also reduce ammonia production due to microbial preference for metabolizing sugars to nitrate compounds (lower energetic consumption). Low mineralization rates are favoring another microorganism’s functional group, involved in atmospheric nitrogen fixation (Boring et al., 1988). The main regulating factors for mineralization are:

- **Temperature:** optimal range between 20-40° (highest rate at 40°);
- **Soil moist:** an elevated soil water content limits mineralization through oxygen depletion;
- **Oxygen availability:** low levels of oxygen reduce mineralization rate, therefore less organic matter is being processed;
- **Organic matter content;**
- **Carbon: nitrogen ratio** within organic matter: optimal levels range from 5:1 – 10:1, and is notable that a ratio higher than 30:1 favors immobilization and a ratio below 20:1 favors mineralization.

### Nitrification

Nitrification is a general process that describes the oxidation of reduced organic and inorganic nitrogen species to nitrite and nitrate. This process requires oxygen and can be accomplished in two ways, depending on the microbial communities. Hence, the first is autotrophic nitrification, mediated by chemolithotrophic bacteria, and the second is heterotrophic nitrification, facilitated by chemoheterotrophic bacteria and fungi.

**Autotrophic nitrification** is a two stage process which corresponds to ammonia reduction to nitrate. The first stage represents ammonia oxidation to nitrite and is accomplished by bacteria such as *Nitrosomonas sp*, during which hydroxylamine is an intermediate compound (eqn [3a], [3b]).

\[
\begin{align*}
\text{NH}_3 + \text{O}_2 & \xrightarrow{\text{Ammonia monoxygenase}} \text{NH}_2\text{OH} + \text{H}_2\text{O} \\
\text{NH}_2\text{OH} + \text{H}_2\text{O} & \xrightarrow{\text{Hydroxylamine reductase}} \text{NO}_2^- + 5\text{H}^+
\end{align*}
\]

(3a)  
(3b)

The second stage represents the further oxidation of nitrite to nitrate, mediated by bacteria pertaining to *Nitrobacter* genus, and this reaction has a fast rate, which explains the general low levels of nitrite encountered in soils (eqn [4]).

\[
\begin{align*}
\text{H}^+ + \text{NO}_2^- + \text{H}_2\text{O} & \xrightarrow{\text{Nitrite dehydrogenase}} \text{NO}_3^- + 3\text{H}^+
\end{align*}
\]

(4)
Autotrophic nitrification is typical for agrosystems, and this process has the potential of reducing the pH, therefore long term use of fertilizers comprise in reduced nitrogen species, often causing soil acidification due to intense nitrification.

**Heterotrophic nitrification** (eqn [5]) represents the oxidation of organic nitrogen and ammonia by numerous heterotrophic bacteria and fungi such as *Arthrobacter*, *Streptomyces* and *Aspergillus*. This process is characteristic for very acid soils in which autotrophic nitrification is practically nonexistent.

\[
\text{organic } N (R - NH_2) \rightarrow NO_2^- \text{ or } NO_3^- 
\]

(5)

Nitrifying microorganisms are susceptible to physico-chemical soil factors such as soil acidity and pH (nitrification is optimal at a pH around 8 and relatively low at a pH below 6), soil aeration, moist and organic matter content, as well as soil texture. The process is promoted by a low C:N ratio.

**Nitrate reduction**

Nitrate and nitrite resulting from nitrification can be reduced in anoxic conditions or soils saturated with water and this process can be accomplished in two ways: denitrification and nitrate dissimilation through reduction to ammonium. This process is opposite to nitrification and it determines nitrogen outputs as molecular nitrogen and nitrogen protoxide.

Biological denitrification is accomplished by heterotrophic bacteria and fungi that utilize nitrogen oxides as electron acceptors (instead of oxygen) and organic carbon as a donor under anaerobic or low oxygen partial pressure circumstances. Denitrification is comprised of a series of stages, each mediated by specific enzymes, and it is noteworthy to mention it is favored by elevated soil organic matter content (eqn [6]).

\[
\text{Nitrate reductase } \rightarrow \text{ Nitrite reductase } \rightarrow \text{ Nitric oxide reductase } \rightarrow \text{ Nitrous oxide reductase } \rightarrow N_2
\]

(6)

Chemical denitrification is soil nitrite reduction through non-enzymatic mechanisms and is noticeable in acid soils (pH ≤ 5), where nitrite accumulation occurs.

The denitrification process is stimulated by high nitrate levels, readily decomposing organic matter (organic carbon availability), oxygen air concentration from soil below 10% and a soil temperature between 25-35°C.

**Assimilation (immobilization)**

The main inorganic nitrogen species that can be assimilated by plant roots are nitrate and ammonium, and it is important to point out that there is a competition for these resources between plants and microorganisms, due to the limiting trait nitrogen has. Recent discoveries (e.g. Nasholm et al, 2009) suggest that organic nitrogen compounds can also be assimilated (most likely simple amino acids) without undergoing mineralization, but in this case there is also competition between biological compartments. Nitrogen species distribution within soil is heterogeneous both in time and space, which has led to the development of various assimilation methods.
Plant nitrogen assimilation is an outcome of a synergy of factors such as: soil ammonium and nitrate concentrations, rhizosphere distribution, soil moist and plant species growth characteristics (e.g., van Dobben et al, 2006).

It is assumed that after the assimilation of ammonium by plants, it is incorporated into amino acids to reduce its toxicity, though recent knowledge suggests a fraction of this nitrogen species can be assimilated without undergoing transformations in plant circulatory system. Ammonium assimilation takes place in the root section of plants, and glutamine is one of the first compounds produced, and in this form is later transported and transformed into other amino acids used for protein synthesis.

Nitrate does not hold a toxic impact upon plants and therefore is assimilated as it is, and sometimes is stored for further transformations or as a regulator of osmotic pressure. Nitrate assimilation firstly undergoes reduction to nitrite, which is further reduced to ammonia mediated by nitrate reductase and utilized as presented above. Therefore, the assimilation of nitrate comes with higher energetic costs compared to ammonium. Also it is noteworthy that the presence of nitrite in plants is also temporarily, as is in soils.

Due to the much shorter life span of microorganisms and their great versatility, it is assumed that they are first in line when it comes to inorganic nitrogen assimilation from soils, and plants utilize the remaining soil nitrogen stocks. The exception to this rule is represented by symbiotic plants (symbiosis with nitrogen fixing microorganisms).

Most nitrogen soil transformations are energy consumption processes, therefore carbon dependent; consequently nitrogen and carbon cycles are indissolubly linked. Nitrogen biogeochemical cycle is also dependent on microbial activity, which has specialized groups for converting nitrogen species. Table 1 shows the main regulating factors of nitrogen soil transformations, as well as the relevant parameters to investigate these processes.

**Table 1.** Factors influencing soil nitrogen transformations and parameters required for nitrogen processes investigation

<table>
<thead>
<tr>
<th>Process</th>
<th>Regulating factors</th>
<th>Optimal conditions</th>
<th>Investigation parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Biological fixation</strong></td>
<td>Presence of oxygen inhibits fixation (affects production and activity of enzymatic complex nitrogenase). Also, nitrogenase synthesis and activity is dependent on soil nitrogen levels. Elevated soil nitrogen levels have a detrimental impact on symbiotic plants.</td>
<td>Anaerobic conditions</td>
<td>Soil aeration Soil nitrogen levels</td>
</tr>
<tr>
<td><strong>Mineralization</strong></td>
<td>Temperature: optimal between 20-40°C. High soil water content is negatively influencing mineralization by depleting available oxygen. Oxygen availability: low levels reduce mineralization rates. Organic matter C:N ratio should be less than 20:1 (a ratio &gt; 30:1 favors immobilization).</td>
<td>40°C Aerobic conditions Organic matter C:N ratio: 5:1 – 10:1</td>
<td>Temperature Soil aeration Soil moist Organic matter content Organic matter C:N ratio</td>
</tr>
<tr>
<td>Assimilation</td>
<td>Occurs when not enough bioavailable nitrogen is present.</td>
<td>Organic matter C:N ratio &gt; 30</td>
<td>Organic matter C:N ratio</td>
</tr>
<tr>
<td>--------------</td>
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</tr>
<tr>
<td>Nitrification</td>
<td>Soil acidity and low pH reduce nitrification rates. Soil physico-chemical characteristics: aeration, texture, organic matter content and moist. Nitrification is inhibited by a pH lower than 6 and it is favored by a low organic matter C:N ratio. Soil oxidation potential positively influences nitrification rates if greater than approximately 400mV.</td>
<td>pH = 8</td>
<td>Soil ammonium&lt;br&gt;Soil aeration and respiration&lt;br&gt;Soil texture&lt;br&gt;Soil moist and oxidation potential&lt;br&gt;pH</td>
</tr>
<tr>
<td>Denitrification</td>
<td>The highest ranking regulating factor is oxygen availability. Low nitrogen and carbon availability is detrimental to denitrification and it can lead to the accumulation of intermediate compounds. Soil nitrate levels and easily decomposing organic matter are also important and higher levels stimulate the process. Temperature influences microbial activity.</td>
<td>Soil nitrate&lt;br&gt;Soil air oxygen concentration &lt; 10%&lt;br&gt;Temperature: 25-35°C</td>
<td>Soil nitrate&lt;br&gt;Soil aeration and respiration&lt;br&gt;Soil texture&lt;br&gt;Soil moist and oxidation potential&lt;br&gt;pH</td>
</tr>
</tbody>
</table>

3. Nitrogen deposition impacts on terrestrial biodiversity

The intensification of nitrogen inputs via atmospheric deposition represents a potential threat to all types of ecosystems and on the quality and quantity of the resources and services they provide (Wamelink et al., 2007). The impact of atmospheric nitrogen deposition alone is difficult to assess due to other contributing factors that manifest simultaneously, such as land use change and global warming. The assessment is increasingly difficult due to the lack of data and information regarding the ecosystem functioning and services before the occurrence of this type of impact. Nevertheless, studies documenting monitoring and experiments of this issue prove to be important analysis instruments which provide useful information concerning nitrogen deposition impacts on biodiversity components.

Several studies demonstrated species richness reduction as a consequence of increased nitrogen deposition. As a result of biological diversity loss, key ecosystem processes and services are altered or lost, such as: productivity, carbon sequestration, nitrogen retention and cycle. It is well proven that the quality of ecosystems services as well as the economic value they attain is strictly dependent on intrinsic ecosystem traits as well on an adequate management of ecosystem processes and efficiency (after Dominati et al., 2010).

Key aspects of the aforementioned approach attempt to respond to the following questions:

- How are nitrogen depositions affecting the structure and functionality of different habitats?
Nitrogen deposition impact on terrestrial ecosystems

- Which is more important: the nitrogen deposition species (oxidized versus reduced) or the type of atmospheric deposition (dry or wet)?
- To what extent are the impacts of nitrogen deposition reversible?
- What management measures can be undertaken and what is their efficiency in maintaining a favorable state?
- How profound is the link between nitrogen deposition and global warming?

A recent review (Bobbink et al., 2010) assesses the effects of atmospheric nitrogen deposition upon terrestrial biodiversity taking into account short term impacts as well as long term detrimental effects (Figure 1).

**Figure 1.** Scheme of the main impacts of increased N deposition on terrestrial ecosystems. Up arrows in boxes indicate increase; down arrows indicate decrease. Thin arrows between boxes mean the effect will occur in the short term (5 years); thick arrows indicate long-term impact; dotted arrows suggest unknown potential impact. Feedbacks are positive (+) or negative (-) (Bobbink et al., 2010)

In arctic and alpine systems the general response to increased nitrogen depositions is the reduction of bryophyte populations while gramineous plants widen. In boreal forests, nitrogen depositions determine major changes of plant associations, without reducing species richness. Bryophytes species as well as lichens and shrubs are disrupted by increased bioavailable nitrogen, while this change favors gramineous and herbaceous species that have higher nitrogen demands (Salemaa et al., 2008). In temperate forests, biological responses to increased nitrogen inputs include an initial growth succeeded by species richness reduction.
due to loss of species adapted to low levels of nitrogen and a decline of species equitably by increased abundance of nitrophilous species.

Within Central Europe and Great Britain, shrub species are positively influenced by nitrogen depositions, but lichen and bryophytes are sensitive to nitrogen enrichment. Excessive grazing as well as drought can manifest synergistically and accelerate grassland species richness loss due to nitrogen enrichment, especially plant species with little coverage. Nitrogen deposition impacts on Mediterranean plant species have been less studied, but several reports also suggest the invasive potential of herbaceous species along with lichens susceptibility to excess nitrogen.

Types of impact

The series of effects following increased nitrogen inputs to system historically adapted to low levels of nitrogen availability is complex. Most ecological processes interact with each other and have different time and space traits. Though the degree of complexity of nitrogen impacts on biodiversity is elevated, these effects can be grouped in general categories for all terrestrial systems.

(a) Direct impact on foliar system

One of the first important impact of nitrogen compounds (gaseous, aerosols and dissolved in precipitations) on above ground plant parts is direct toxicity. Nitrite, ammonium and particularly ammonia are phytotoxic nitrogen species. These effects have mainly been studied in crops, young trees and native species and demonstrated foliar impacts, physiological changes and growth limitation due to high levels of nitrogen compounds. It has been showed that lichens are most sensitive to ammonia dry depositions, and that bryophytes are susceptible to low wet nitrogen depositions, therefore these species are generally used as indicators of increased nitrogen depositions (Sutton et al., 2004, Britton et al., 2010).

(b) Eutrophication

Nitrogen is a limiting factor of plant species growth in most natural and semi natural systems, which are generally oligotrophic and mesotrophic. Elevated nitrogen deposition leads to topsoil temporarily increase of available nitrogen. Subsequently, productivity and litter production increase, which determines the intensification of the mineralization process that furthermore stimulates productivity. However, after a certain value of primary production, local plant species diversity declines, favoring species adapted to enriched nitrogen soils (nitrophilous).

(c) Acidification

Soil acidification is the loss of buffering capacity and pH reduction. In calcareous systems, initial stages do not include acidification following acid depositions, but the soil is depleted of calcium and carbonic ions and the pH starts to decline after the loss of calcium carbonates. In soils dominated by mineral silicates (pH between 6.5-4.5) buffer capacity depends on cation exchange capacity. In this case protons are substituted with calcium and magnesium ions that are percolated afterwards along with anions (generally nitrate and sulphate). Due to limited buffering capacity of these systems, pH decline is more rapid compared to calcareous soils. Mineral soils with elevated cationic exchange capacity and rich in basic compounds can maintain an adequate pH level for longer time periods (decades), even if acidic inputs are constantly high. A pH below 5 allows mineral constituents decomposition, which
subsequently leads to hydroxides and metals dissolution. Under these circumstances, a significant accumulation of toxic metals occurs, especially aluminum. The decline of pH limits or inhibits nitrification, which allows ammonium build up and decrease of nitrate concentrations. In acidified soils, mineralization rates reduce, therefore litter accumulation is elevated. As a result, plant species growth and species composition is significantly impacted: acid loving plants are favored and become dominant, whereas species adapted to neutral or basic pH levels are lost.

The profundity of nitrogen deposition impacts is influenced by various factors, the most significant being:

- Exposure duration and amount of deposited nitrogen;
- Type of nitrogen deposition and species;
- Intrinsic sensitivity of plant and animal communities;
- Abiotic factors;
- Past and present land use and management.

Other important factors that influence nitrogen terrestrial processes are soil buffering capacity, initial nutrients bioavailability (nitrogen and phosphorus) and soil physical and chemical properties (Erisman et al., 1995, Bobbink and Hicks, 2009).

Present global warming and atmospheric carbon dioxide enrichment determine a higher nitrogen plant uptake, therefore is favoring species growth (van Dobben et al., 2006). However, temperature increase also intensifies mineralization rates which regulate soil nitrogen percolation, hence soil nitrogen stocks reduce and nitrogen outputs further impact other biodiversity components. Therefore, global climatic trends have the potential of intensifying the impacts generated by nitrogen depositions. The previous premise is endorsed by a study on modeling acid atmospheric depositions and global climatic trends carried out by Sanderson et al. (2006).

The investigation of nitrogen species ratio (oxidized versus reduced) from atmospheric nitrogen deposition is relevant due to distinct emission sources (agriculture for reduced nitrogen species and industry and transport for oxidized nitrogen species). Similarly, plant radicular system is exposed to different ratios and concentrations of reduced and oxidized soil nitrogen species. Therefore, alkaline habitats are less susceptible to elevated ammonium levels compared to those with acidic soils (Stevens et al., 2011a, 2011b).

4. Trends and evolution of studies regarding nitrogen deposition impacts on terrestrial systems

Atmospheric deposition monitoring

The vast majority of monitoring studies on atmospheric nitrogen and succeeding impacts have been achieved at European scale (mostly Great Britain and Holland), United States of America and parts of Asia.

In Great Britain, information regarding precipitations’ chemical composition exist as far back as 19th century due to raising concerns on atmospheric pollutants emissions from households and industry (Metcalfe et al, 1998, Fowler et al., 2005). In 1996, a precipitation chemical composition network was established, as well as a monitoring network for sulfur dioxide emissions from rural areas. Between 1986 and 2000, a 72% decrease of sulfur emissions has been reported, as a result of implementing national and international directives regarding Romanian Biotechnological Letters, Vol. 18, No. 6, 2013
emission reduction (e.g. Convention on Long-range Transboundary Air Pollution). Furthermore, the precipitation acidifying potential has halved, though nitrogen species showed a smaller reduction and only at a micro regional scale (Fowler et al., 2005). Recent studies (Matejko et al., 2009) report that sulfur dioxide emissions decreased by 89% in 2005 compared to 1970, primarily due to technological progresses (the use of natural gas in lieu of fossil fuels, as well as installation of emission reduction equipment in electric plants). The latter study suggests that during the same interval (1970-2005), oxidized nitrogen emissions dropped by 48%.

Stevens et al. (2009) have studied soil acidification and fluctuations of ionic metals with exchange capacity potential as a result of acidifying atmospheric depositions. The most statistically relevant parameters in explaining acidic deposition effect were (highest ranking to lower ranking): soil pH (negative correlation), topsoil C:N ratio (negative correlation), latitude and slope (negative correlations). By distilling the effects determined by nitrogen and sulfur, it was determined that atmospheric nitrogen deposition have a more significant influence on soil pH variations (R^2=0.32) than sulfur depositions (R^2=0.22), which suggests a higher acidifying effect of nitrogen.

Russia has also a historical interest in precipitation chemical composition that dates more than a century ago, due to interest regarding soil nutrient inputs (Ryaboshapko et al., 2010). Therefore, an atmospheric composition monitoring network was established a few decades ago. Similarly, a significant reduction of emission was reported for sulfur compounds during 1990-2004 (almost threefold), followed by nitrogen compounds reduction (1.4 times).

Ying et al. (2006) have studied spatial and temporal distribution of atmospheric nitrogen depositions in North China Plain in order to provide information at a national level (studies regarding this analysis were scarce). It was reported that annual nitrogen inputs were elevated enough to characterize the impact system as “polluted” and that 60% of total atmospheric nitrogen depositions occur during warm season, from June to September. Nitrogen atmospheric deposition monitoring at a national scale begun during late 90s (Liu et al., 2011), and data provided was insufficient to adequately assess the impact of excess nitrogen inputs. The economic growth of this region is presumed to lead to an intensification of nitrogen emissions, mainly stemming from agriculture, industry an urban areas. Results regarding oxidized and reduced nitrogen species from PM10 (particulate matter with more than 10 microns in diameter) suggest there are nitrogen pollution “spots”, such as large cities and agricultural areas (e.g. the North China Plain). Another study carried out by Haiping et al. (2011) highlighted the impact of nitrogen atmospheric depositions on phytoplankton communities, their biological response consisting in a 30.6% growth in a few days following precipitations rich in nitrogen compounds.

In USA, the highest values for nitrogen atmospheric deposition are cumulated in the Western region, mainly urban areas and farms or downwind from agricultural systems (Holland et al., 2005). In these areas, nitrogen depositions can be over 20 kg/ha/year (Fenn et al., 2006).

More subtle effects of nitrogen enrichment such as acidification and impact on biotic compartments (such as lichens and diatoms) have been reported for distant areas, far from emission sources. These impacts can occur at low levels of atmospheric nitrogen deposition, between 3-8 kg N/ha/year (Fenn et al., 2006). Another study carried out by Douglas (2003) has illustrated nitrogen saturation for alpine systems from Rocky Mountains, though deposition were only as high as 6.4 kg N/ha/year. The synergic effect of ozone and nitrogen
depositions has also been reported by Fenn et al. (2006), that documented major changes at a plant physiological level, nutrient cycling, carbon sequestration and invasive insects attacks.

**Experimental nitrogen addition studies**

Numerous experimental nitrogen addition studies have been carried out in order to observe and explain terrestrial systems responses to excess reactive nitrogen inputs. These studies need to meet different sets of criteria, depending on established objectives (e.g. response mechanisms, competition for nutrient stocks). Consequently, data analysis is performed taking into account selected criteria (such as: type of fertilization, chemical species used, addition frequency and intervals, spatial scale and presence of management activities). The majority of this analysis instruments have been dedicated to soil and plant cover compartments, though there are some studies that have taken into account the faunal compartment. Murray et al. (2006) have demonstrated nematodes population reduction following nitrate and calcium carbonate addition. Guo-Liang et al. (2007) have indicated that for sub-tropical systems, low levels of nitrogen addition are beneficial (favoring soil fauna), whereas levels of deposition greater than 100 kg N/ha/year are detrimental for soil faunal compartment. Bai et al. (2010) state that there is a limited range of nitrogen addition that can favors productivity without affecting biodiversity. Also, biological responses prove to be a better and ecological significant indicator of nitrogen deposition impacts. Most experimental studies have used increased levels of fertilizations to reduce the temporal scale in order to attain more relevant results.

The value of aforementioned type of studies can represent at least two knowledge categories: firstly they corroborate the causal relationship between nitrogen deposition and biodiversity loss, secondly they provide useful information for designing scenarios of further nitrogen depositions impact on terrestrial ecosystems by extrapolating experimental findings to different time and space scales. Cunha et al. (2002) have synthetized the experimental nitrogen addition results according to analyzed types of ecosystems, detailed below.

**Forest ecosystems**

Nitrogen addition experiments have resulted in changes in trophic state of tree species, as well as a growth or decline stimuli (depending on the levels of surplus nitrogen). Plant cover diversity (bryophytes and higher plants), as well as mycorrhizal abundance and diversity have reduced under excessive nitrogen inputs, most likely due to nitrogen being a limiting factor (e.g. Bradley et al., 2006). Growth has been reported for nitrophilous species, mainly herbaceous species. Other documented responses were litter production, pathogenic fungal growth, as well as insect pests that are detrimental for plant cover and trees. The soil responses to increased nitrogen availability included: intensified nitrate, ammonium and basic cations percolation rates, along with soil ammonium enrichment.

**Dune and bog ecosystems**

Though these ecosystems are different both pedologically and climatically, similar responses have been illustrated after experimental increased nitrogen inputs, the general trend being conversion to acidic grasslands. Short term studies proved that nitrogen enrichment favors these systems, but long term fertilizations proved adverse effects. Opposite to forest ecosystems, percolation rates did not increase, even under very elevated nitrogen depositions, which suggests soil nitrogen immobilization.

**Grasslands**
Biodiversity in grasslands is higher compared to other types of ecosystems, therefore most grasslands have been converted to arable lands. In grassland ecosystems, nitrogen addition responses are directly influenced by soil pH (calcareous or acidic systems) and include changes in species composition and soil and plant chemistry (e.g. Langan et al., 1994). Acidic grasslands are more vulnerable to elevated nitrogen inputs (especially reduced nitrogen species) while calcareous grasslands are rather phosphorus limited and have a better buffering capacity for acidifying inputs. Therefore a combination of nitrogen and phosphorus fertilization can affect at a greater rate calcareous grasslands than the addition of nitrogen alone.

Plant cover changes induced by reactive nitrogen enrichment are typically the decline of lichen and bryophyte populations (e.g. Wilson et al., 2009) and other nitrogen sensitive species in favor of herbaceous species characteristic for acid soils, such as: *Festuca ovina* and *Arrhenatherum elatius*. Subsequently species richness is lost. Also, changes in nutrient cycling have been demonstrated after bioavailable nitrogen enrichment such as mineralization intensification, biomass accumulation and microbial activity increase (e.g. Horswill et al., 2008). Negative effects of nitrogen addition on soil buffering capacity have also been studied by Bowman et al. (2008), and noted up to 38% decline of basic cations involved in soil buffering capacity after experimental nitrogen addition. To better illustrate differences between calcareous and acidic grasslands, Lee and Caporn (1998) compared the two types, both untreated and fertilized with 14 g N/m²/year, and analyzed dry and wet deposition (as nitrogen inputs) and mineralization rates. They documented increased mineralization rates for acidic grasslands and significant intensification of organic matter decomposition after nitrogen addition.

Allen et al. (1999) analyzed results from famous fertilization experiment carried out by Rothamsted Experimental Station (present nomenclature: Rothamsted Research), where the same plots were continuously fertilized with ammonium for more than a century (120 years) at a deposition rate of 144 kg/ha/year (Marrs and Gough, 1989). The authors noted a radical species richness loss, from 17 to only 2: *Alopecurus pratensis* and *Anthoxanthum odoratum*. Another notable study discussed by Allen et al. (1999) was conducted by Tilman, which encompassed various fertilization rates (10 – 272 kg N/ha/year) during 4 years interval. In this case, 60% of species diversity was lost and an invasive species (*Agropyron repens*) acquired dominance. Both studies strongly indicate that along with elevated nitrogen inputs, plant associations are significantly affected.

Another study carried out by Allen et al. (2009) in Joshua Tree National Park (USA) analyzed plant associations’ responses after experimental nitrogen addition. Study premises includes species susceptibility to nitrogen depositions, as well as increased productivity and habitat expansion of three invasive species (*Schimus barbatus*, *Bromus madritensis* and *Erodium cicutarium*) for the last two decades prior to the study. An experimental nitrogen fertilization at rates between 5 – 30 kg N/ha/year carried out for two years was implemented in order to confirm the hypothesis that aforesaid changes occurred as a result of atmospheric nitrogen depositions. A biomass increase of the invasive species was demonstrated at highest fertilization rates. Though current nitrogen deposition rates were considerably below 30 kg N/ha/year, it is highly probable that in time bioavailable soil nitrogen stocks will increase due to low percolation rates characteristic to study area and that will favor the dominance of invasive species.

Biomass changes as a result of nitrogen enrichment have also been reported by Lee et al. (2010), with the notable observation of change of the shoot/root ratio (ratio increases). Another study that documented radicular system reduction as a consequence of soil increased
nitrogen stocks was carried out by Zeng et al. (2010) in a grassland ecosystem from North-East China. In this study, the shoot/root ratio halved.

Atmospheric nitrogen impact on forest ecosystems

Atmospheric nitrogen deposition generate various changes to forest ecosystems and due to physical structure (roughness), they possess the ability to intercept higher levels of depositions compared to flat areas (2 - 3 times more). Negative impacts occur on plant species composition and distribution, as well as on mychorizzal communities and soil biogeochemical cycles. Elevated nitrogen inputs lead to soil acidification and nitrogen percolation rates. Observable effects are prominent under high nitrogen deposition rates over a prolonged time frame. Forest ecosystems are nitrogen limited, therefore initial nutrient surplus will induce positive effects such as increased tree species growth and development, resulting in greater timber production and carbon sequestration. These benefits are only temporarily, since forthcoming soil acidification and nitrogen saturation have proven to be detrimental. For instance, nitrogen percolation rate can be as high as 100% under nitrogen depositions greater than 40 kg N/kg/ha/year (ICP Forests, 2011). Atmospheric nitrogen depositions vary from 5 kg/ha/year in Northern Europe and over 60 kg/ha/year in Western and Central Europe and forest plant cover intercept approximately half of these inputs (Butterbach-Bahl et al., 2011).

Lorenz et al. (2010) investigated nitrogen mobilization within a forest ecosystem from North-Western Germany, which during 1998-2008 received elevated nitrogen wet depositions, between 23-35 kg/ha/year. Their objective was the study of increased nitrogen input effects on nitrification and ammonification, as well as the risk of elevated nitrogen outputs through percolation. Their results suggest that ammonium has highest mobility, which directly affects nitrification. A positive correlation was also identified between ammonification and ammonium atmospheric depositions. Low pH levels along with increased atmospheric depositions are key factors that turn this ecosystem types into sources of nitrogen for other systems due to increased nitrogen outputs via percolation.

Van Dobben and de Vries (2010) statistically demonstrated that, along with general traits (soil characteristics, climatic regimen and tree species) that directly influence the composition of plant associations, atmospheric nitrogen depositions are also a key factor.

Atmospheric nitrogen impact on grassland ecosystems

Most plant associations from semi natural grasslands are dominated by nitrogen limited species, therefore they are sensitive to acidification and eutrophication (e.g. Hall and Wadsworth, 2010). Nitrogen deposition impacts on these systems are a result of the amount of nitrogen input via atmospheric deposition, exposure duration, soil buffering capacity and land use type (Dise, Stevens, 2005). It was demonstrated that in acidic grassland, a species is lost every 2.5 kg N/ha/year. Due to the high complexity degree of these factors at a macro and micro regional scale, grassland susceptibility varies within an extended interval (Bleeker et al., 2011), most vulnerable being grasslands with acidic soils.

A comprehensive study carried out by Stevens et al. (2010) at Atlantic Europe bioregion scale indicates a species richness reduction of acidic grasslands as a direct result of elevated nitrogen atmospheric depositions. Study areas were selected in regions from Great Britain, Ireland, France, Germany, Holland, Denmark, Sweden and Norway. The study took place during 2002-2007, and selected ecosystems were not fertilized, most of them being included in protection and conservation areas. The range of nitrogen atmospheric depositions was 2-44 kg/ha/year, and a negative correlation was established between species richness and level of deposition. This relation is highly important for environmental policies concerning...
biodiversity protection and suggests the need for increased protection particularly for less impacted systems. The obtained results of this study also demonstrate that the impact of nitrogen deposition can be found at large spatial scales, in this case at macro regional level. Though species richness loss was the primary observation of nitrogen deposition impact on biodiversity, the species number alone is not always relevant, species identity can prove to be more relevant. Bobbink et al. (2004) suggested that species richness can vary, but they can also be replaced by other species, therefore a relevant approach is the analysis of species composition and induced structural modification as well.

5. Management of nitrogen deposition impact on terrestrial biodiversity

Fenn et al. (2010) developed a series of management actions that can be implemented to mitigate the impact of nitrogen deposition impact on terrestrial ecosystems which are grouped in four major categories:

1. Nitrogen inputs reduction;
2. Increase of nitrogen uptake rates;
3. Increase nitrogen outputs by harvesting plant material;
4. Restoration of soil nitrogen retention capacity.

Most management actions concerning mitigation of nitrogen deposition impacts assume the reduction of carbon and other nutrients along with nitrogen, which can be unfavorable for ecosystem functioning. Therefore, the most efficient preservation activity is depositions reduction, which translates into emissions reduction, most effectively at a regional scale due to long distances of atmospheric transport. Also, it is very important to take into account soil pH, inextricably linked to ecosystem susceptibility to nitrogen deposition impacts (e.g. Pitcairn et al., 2006). Another promising solution is carbon addition, the purpose being increasing C:N ratio, therefore stimulating nitrogen immobilization, ultimately reducing nitrophilous invasive species occurrence. It has been stated that carbon addition rather reduces nitrate than ammonium (Eschen et al., 2007). For less acidic soils, in which main threat is acidification, calcium oxide addition proved to be beneficial (Stevens et al., 2011a, 2011b). In basic soils, light availability is the key limiting factor, which suggest grazing or increased harvest frequency as potential managerial activities.

6. Conclusions

The most relevant conclusions of the studies concerning nitrogen depositions impact on terrestrial ecosystems can be summarized as follows:

Main effects of increased nitrogen inputs are: soil acidification, soil basic cations loss, eutrophication, and biological changes such as increased shoot/root ratio. These chemical and biological effects are core changes that further determine biodiversity loss;

Soil basic cations loss as a result of acidic and nitrogen depositions exceeding nitrogen saturation results in nutrient imbalances for most ecosystems, directly impacting ecosystem health and stability. As a general trend, studies regarding atmospheric nitrogen deposition on forest ecosystems illustrate a 10% decline for more than two thirds at European scale, mainly triggered by ammonia and nitrogen oxides emissions.

Significant changes of bioavailable nitrogen occur at depositions greater than 100 kg/ha/year, whereas biological responses take place at much lower levels, approximately 20-50 kg/ha/year;
Lichen species are most sensitive indicators of ecological impacts, including nitrogen depositions. Near 80% of studied areas showed changes in lichens population as a result of increased nitrogen inputs. Plant species are not the only biological indicators of nitrogen deposition impact, microbial communities having a more rapid response and it has been documented that faunal responses (soil macroinvertebrates) are also significant;

Nitrogen critical load exceedance has reduced as a result of implementing environmental policies, but levels continue to remain elevated, further emission reductions being imperative.

Soil acidification continues to be problematic, systems recovery require decades to limit the impact. Species richness loss has profound implications on biodiversity, ecosystem functioning, quality and quantity of provided resources and services.

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