

NITROGEN DYNAMICS AND INDICES TO PREDICT SOIL NITROGEN SUPPLY IN HUMID TEMPERATE SOILS

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Abstract

Knowledge of the nitrogen (N) available to crops during the growing season is essential for improving fertilizer-use efficiency and minimizing the adverse impacts of N losses on the environment. In humid temperate regions, soil N supply is dominated by in-season N mineralization because plant-available

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N ($\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$) is transformed to nonlabile forms or lost from the soil-plant system during fall and winter. The microbially mediated reactions that generate the soil N supply in agroecosystems are affected by system-specific conditions, including soil properties, agricultural management (crop rotation, tillage system, organic amendments), and most importantly, climate. Potentially mineralizable N (N_o) determined from long-term soil incubation is regarded as the standard measure of soil N mineralization potential and may provide a good approximation of the soil N supply. However, this method is time consuming and not practical for routine use. Several chemical methods to estimate the N mineralization potential of soils are discussed in this chapter. The major limitation of chemical methods is that they cannot simulate the microbial-mediated release of plant-available N under field conditions. Consequently, any single chemical method may not be a good predictor of soil N supply. Thus, we suggest a holistic approach to estimate soil N supply in humid temperate regions, which involves (1) the use of a combination of N indices together with weather data and (2) identification and quantification of a specific fraction (s) of organic N that is the dominant contributor (s) to N supply in a particular system.

1. INTRODUCTION

Nitrogen (N) is the most limiting nutrient for crop production in humid temperate soils, although the total N content in these soils can be quite appreciable (e.g., 0.48–3.36 g N kg⁻¹ in meadow soils in Quebec, Canada; [Simard and N'Dayegamiye, 1993](#)). Organic N forms constitute up to 90% of the total N in the plow layer of mineral soils ([Olk, 2008b](#)), and only about 1–4% is mineralized as plant-available N ($\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$) each year ([Tisdale *et al.*, 1985](#)). Mineralization of organic N is seldom sufficient to achieve the maximum yield possible with modern cultivars, so additional N from mineral fertilizer or organic sources (e.g., animal manures and legume residues) is needed. Plant-available N released from soil organic N (SON) or applied in fertilizer is highly susceptible to loss from the soil-plant system through leaching and denitrification. This means that N management is of great importance for both economic and environmental reasons. Optimizing N management will improve agronomic performance and increase economic returns, as well as reduce N losses to the environment. One approach to reduce environmental pollution and improve profitability is to tailor fertilizer N applications to meet N crop requirement ([Sharifi *et al.*, 2007a](#); [Zebarth and Rosen, 2007](#)) by synchronizing the application of plant-available N with crop N uptake in space and time ([Ma *et al.*, 1999](#)). However, this is difficult to achieve due to substantial variation in both crop N demand and in soil N supply across years and within and between fields ([Zebarth *et al.*, 2009](#)). In arid and semi-arid environments, soil N supply consists of mineralization of SON, crop

residues, and other organic amendments plus residual soil mineral N from the previous growing season. However, in humid temperate regions, N supply consists primarily of in-season N mineralization due to significant N losses during fall and winter (Zebarth *et al.*, 2009). Although considerable progress has been made in understanding the controls of N availability in crop production systems, we still do not have a soil N test that can reliably predict the N supply in humid temperate regions.

This chapter discusses soil N dynamics, current measures for estimating soil N supply, and suggests a holistic approach to predicting N availability in cropland of humid temperate regions.

2. NITROGEN DYNAMICS

2.1. Importance of nitrogen in crop production

Nitrogen occupies a unique position among essential elements because agricultural crops require proportionally more N than other nutrients to achieve maximum yield. For instance, fertilizer recommendations in Quebec, Canada are 120–170 kg N ha⁻¹ and 20–95 kg P₂O₅ ha⁻¹ for corn (*Zea mays* L.), and 40–120 kg N ha⁻¹, 20–80 kg P₂O₅ ha⁻¹, and 20–90 kg K₂O ha⁻¹ for wheat (*Triticum aestivum* L.; [Centre de référence en agriculture et agroalimentaire du Québec, 2003](#)). Nitrogen is considered the most important growth-limiting factor in nonlegumes (cereal crops; Zebarth *et al.*, 2009). Plants that have not received sufficient N exhibit stunted growth and gradual leaf yellowing but respond quickly to N inputs (e.g., leaves turn deep green in color due to the central role of N in chlorophyll synthesis). As few soils supply enough N to sustain satisfactory crop production without fertilizer, N deficiency is more widespread than any other nutrient deficiency in crops.

Rapid increases in crop yields became possible with the introduction of mineral N fertilizers. The positive effect of mineral N fertilizer application on yield is well documented for crops grown in humid temperate regions, such as potato (*Solanum tuberosum* L.; [Bélanger *et al.*, 2000](#); Zebarth and Rosen, 2007; Ziadi *et al.*, 2011), wheat (Holford and Doyle, 1992; Westerman *et al.*, 1994), barley (*Hordeum vulgare* L.; Zebarth *et al.*, 2008), corn (Gagnon and Ziadi, 2010; Halvorson and Reule, 2006; Stanger and Lauer, 2008), and forage grasses (Ziadi *et al.*, 2000). Fertilizer N contribution to total N inputs into agricultural systems rose from just 7% in 1950 to 43% by 1996 (Mosier, 2001) and should continue to increase, especially in areas where the arable land base is shrinking and the goal is to increase output per unit area through more intensive agricultural management (Mosier, 2001). Improvement of N-use efficiency from fertilizer is critical since the average recovery of added N is about 50% globally (Smil, 1999) and overapplication

of fertilizer can result in N losses from the soil–plant system that affect surface and groundwater resources (Fageria and Baligar, 2005).

2.2. The global nitrogen cycle

The global N cycle (Fig. 1) describes the principal pools and forms of N, and the processes by which N is transformed in terrestrial ecosystems. Nitrogen exists in the atmosphere as dinitrogen gas (N_2), oxides (N_2O , NO , and NO_2), and in the reduced form (NH_3). These N forms also exist in soil water as dissolved gases. Hart *et al.* (1994) explained that the biogeochemical cycling of N in terrestrial ecosystems can be divided into an external and internal cycle. The external cycle includes processes that add N to ecosystems (N_2 fixation, ammonium (NH_4^+) and nitrate (NO_3^-) in rainwater, mineral and organic N fertilizer applications) and cause N loss from ecosystems (denitrification, NO_3^- leaching, and NH_3 volatilization). In contrast, the internal N cycle consists of processes that convert N from one chemical form to another or transfer N between ecosystem pools including plant assimilation, return of N to the soil in plant litter-fall and root turnover, N mineralization, and microbial immobilization. The entrance, removal

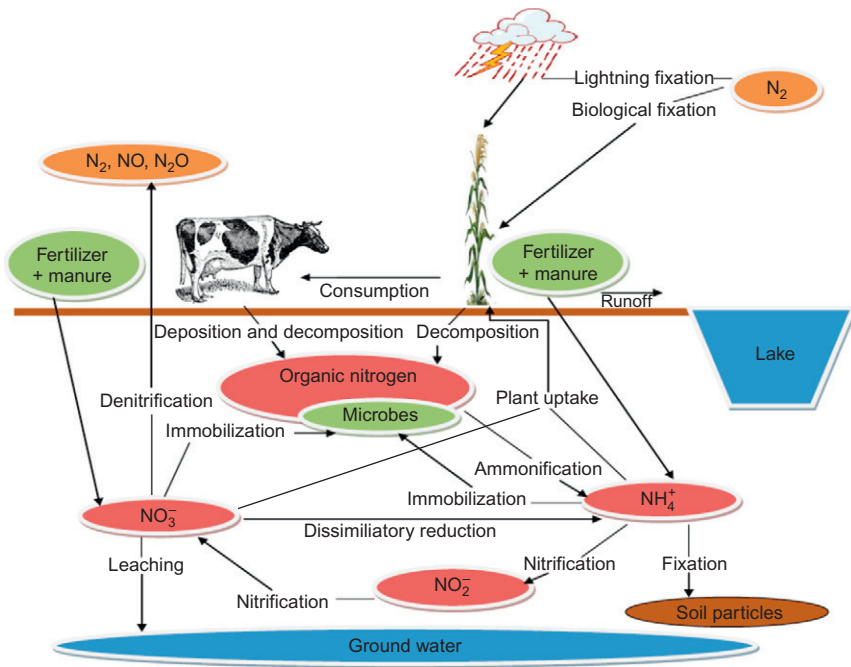


Figure 1 Illustration of the nitrogen cycle in soil.

and/or lost and subsequent reentry of N between the soil and atmosphere complete the global N cycle.

2.3. Nitrogen cycling in the plant–soil system

Nitrogen cycling in the soil–plant system is very complex and involves interactions between soil and plant factors. The major N transformation processes in this system in humid temperate soils are mineralization, nitrification, immobilization (refer to [Section 2.5.1](#)), and denitrification (refer to [Section 2.6.1](#)). The mineralization of organic N compounds into inorganic N (considered the major source of plant-available N) generates the soil N supply; it is also an important starting point for potential losses of N and attendant adverse environmental impacts ([Myrold and Bottomley, 2008](#)). Mineralization occurs largely through biological activities that are temperature and moisture dependent ([Agehara and Warncke, 2005](#); [Stevenson, 1986](#)). Recently, [Kemmitt *et al.* \(2008\)](#) suggested that N mineralization is not regulated by the size, composition, or activity of the soil microbial biomass, rather, an abiological process termed the “regulatory gate” controls the rate-limiting step of N mineralization. This has triggered an interesting debate among researchers ([Brookes *et al.*, 2009](#); [Kuzyakov *et al.*, 2009](#)).

The N mineralization process consists of sequential aminization and ammonification reactions. Aminization requires extracellular enzymes of bacterial and fungal origin such as proteinases and proteases that break down complex proteins into simpler amino acids and amino sugars ([Myrold, 2005](#); [Whalen and Sampedro, 2010](#)). These small organic N compounds are further hydrolyzed within microbial cells by intracellular enzymes such as arylamidase and amidohydrolase during ammonification to yield NH_4^+ . The amine ($\text{NH}_2\text{-CR}_3$) and amide ($\text{NH}_2\text{-CR=O}$) functional groups are two N forms in amino acids that undergo degradation. Amide groups are cleaved by specific enzymes, for instance, asparagine and glutamine are hydrolyzed by asparaginase and glutaminase. Amino-N is released by amino acid dehydrogenases and amino acid oxidases by deamination ([Myrold, 2005](#)). Amino sugars are metabolized in two steps. They are phosphorylated by a kinase, and then NH_4^+ is released through deamination ([Myrold, 2005](#); [Whalen and Sampedro, 2010](#)).

Humid temperate soils often have low NH_4^+ concentration because it is rapidly converted into NO_3^- through nitrification by chemoautotrophic microorganisms ([Whalen and Sampedro, 2010](#)). This reaction often limits the amount of NH_4^+ available for plant uptake following the application of NH_4 -based fertilizers ([Subbarao *et al.*, 2006](#)). However, the nitrification rate can be reduced by banding of NH_4 -based fertilizers due to high osmotic pressure or high pH in the bands ([Malhi and Nyborg, 1988](#); [Wetselaar *et al.*, 1972](#)) and toxicity of high NH_3 concentration ([Malhi and McGill, 1982](#)). Nitrification involves three key reactions. Ammonium is first oxidized to

hydroxylamine and then to nitrite (NO_2^-) by ammonia-oxidizing bacteria (AOB) and archaea (AOA), and finally to NO_3^- by nitrite-oxidizing bacteria (Whalen and Sampedro, 2010). The advent of advanced molecular techniques such as quantitative real-time polymerase chain reaction (qRT-PCR) and stable isotope probing has recently illustrated the importance of AOA in soil (Gleeson *et al.*, 2010; Nicol *et al.*, 2008; Treusch *et al.*, 2005). The AOB (*Nitrosomonas* being the most well known) use hydroxylamine oxidoreductase to transform hydroxylamine to NO_2^- . Some AOB also denitrify the NO_2^- to NO , N_2O , and N_2 through the nitrifier-denitrification pathway (Wrage *et al.*, 2001), and therefore cause gaseous N loss from the soil-plant system. Nitrite does not accumulate in soil because it is rapidly transformed to NO_3^- by nitrifiers (*Nitrobacter* being the most well known) that possess nitrite oxidoreductase or other oxidizing enzymes (Whalen and Sampedro, 2010). Heterotrophic microorganisms including fungi (e.g., *Aspergillus*) and bacteria (e.g., *Alcaligenes* and *Arthrobacter* spp.) can also oxidize either NH_4^+ or organic N to NO_2^- or NO_3^- (Myrold, 2005). In addition, other microbes can produce NO_2^- and NO_3^- by enzymatic oxidations that are not linked to microbial growth (Myrold, 2005; Sahrawat, 2008; Whalen and Sampedro, 2010).

2.4. Soil nitrogen supply in humid temperate soils

While biological N_2 fixation makes an important contribution to the N requirements of leguminous crops (about 40–60%; Herridge *et al.*, 2008), nonleguminous crops must absorb plant-available N from the soil, mainly NH_4^+ and NO_3^- from mineralization and nitrification reactions, as well as free amino acids (Adamczyk *et al.*, 2010; Jones *et al.*, 2005; Sauheilt *et al.*, 2009). Plant-available $\text{NH}_4\text{-N}$ must be distinguished from fixed $\text{NH}_4\text{-N}$ bound within the expanding lattices of clay minerals that is either sparingly available or unavailable for plant uptake. Chantigny *et al.* (2004) showed that clayey soils in the humid regions of Eastern Canada were a sink for $\text{NH}_4\text{-N}$ as up to 34% of recently added NH_4 -based fertilizers were fixed in the clay fraction.

In humid temperate regions, most of the plant-available N remaining from the previous growing season is lost from the rooting zone during fall and winter due to high soil moisture. Therefore, soil N supply in these conditions is controlled by mineralization of SON during the growing season (Zebarth *et al.*, 2005, 2009). In some instances, N supplied from SON may exceed the N absorbed from mineral fertilizers. A study with ^{15}N -labeled fertilizer showed that current-year N fertilization contributed only 10–50% of the total N uptake by corn, with the remainder supplied from soil N (Stevens *et al.*, 2005). Wu *et al.* (2008) reported that 87% of the N uptake by corn in Ontario came from N mineralization and nitrification.

2.5. Factors affecting nitrogen supply in humid temperate soils

According to Zebarth *et al.* (2009), N supply is sensitive to the factors that control N mineralization potential (e.g., soil properties and agricultural management) and to environmental conditions (e.g., moisture and temperature) that regulate the biological activity controlling the mineralization process. Hence, soil chemical, physical, and biotic properties, and agricultural practices that affect the concentration and biochemical degradation of SON will affect the soil N supply.

2.5.1. Soil chemical properties

Soil chemical properties including pH, electrical conductivity (EC), cation exchange capacity (CEC), C/N ratio of added amendment, and organic matter (OM) content can impact soil N supply (Dharmakeerthi *et al.*, 2005; Subbarao *et al.*, 2006) by influencing the activity of microorganisms and the concentrations of NH_4^+ and NO_3^- in the soil solution. Many soil microorganisms function optimally in the soil microenvironment with a pH ranging from 6 to 7 since most soil nutrients are available in this range (Hartel, 2005); however, this depends on the microbial group, given that the activity of N-mineralizing bacteria is greatest between pH 6.5 and 8, whereas pH 5.5–6.5 is ideal for fungi (Whalen and Sampedro, 2010). Soil microorganisms responsible for N mineralization are less sensitive to increases in soil pH and EC than nitrifier populations (Griffin, 2008). Thus, nitrification is rapid in soils with a pH ≥ 6.0 but slower in soils with a pH ≤ 5.0 (Sahrawat, 2008; Subbarao *et al.*, 2006). In a 15-day incubation experiment with various soils treated with 100 ppm of $\text{NH}_4^+\text{-N}$ as $(\text{NH}_4)_2\text{SO}_4$, Dancer *et al.* (1973) found a three- to fivefold increase in nitrification as soil pH increased from 4.7 to 6.5. De Neve *et al.* (2003) incubated a loamy sand for 173 days at six different temperatures (5.5–30 °C) and at a constant water content (80%) and reported that the EC of the soil extracts increased significantly with increasing temperature, and was strongly correlated ($r = 0.96$) to N mineralization. Pathak and Rao (1998) measured $\text{NH}_4\text{-N}$ up to EC 70 dSm^{-1} , while $\text{NO}_3\text{-N}$ was undetectable at EC $< 26 \text{ dSm}^{-1}$ in saline and sodic soils. They concluded that net N mineralization decreased with increasing pH or salinity. The CEC of a soil represents its ability to store and release cations such as NH_4^+ into the soil solution for plant uptake. This significantly reduces the potential leaching losses of plant-available N, thus enhancing the N supply capability of the soil. In a 6-week greenhouse experiment involving 21 soils, Abadín *et al.* (2010) reported that mineralized N and N uptake by wheat were significantly correlated to CEC ($r = 0.68$ and 0.60, respectively).

Crop residue quality, related to lignin content and C/N ratio, determines whether microorganisms will immobilize mineral N or release it into

the soil solution, as low N concentration in soil solution limits microbial growth (Henriksen and Breland, 1999). Soil N supply is greatly reduced during the early stages of decomposition of plant residues with high C/N ratios because of the greater N demand by the microbial biomass in response to the input of available carbon (C). Hence, net immobilization occurs when microorganisms assimilate recently mineralized N and inorganic N from the soil solution. Green *et al.* (1995) showed that rapid immobilization of mineral N occurs during the initial decomposition of corn stover. However, as decomposition proceeds, the C/N ratio of the residue becomes lower and the activity and population of soil microbes is reduced due to limited C availability, resulting in the release of mineral N from the both the decomposing residue and dead microbes. It is well known that soils amended with organic materials having a C/N ratio less than 20:1 generally exhibit net mineralization. The C/N ratio of the microbial population is also critical. The C/N ratio of microorganisms can range from 4 to 5 for bacteria, and to as high as 15 for fungi (Myrold, 2005). As fungal biomass contains more C per unit N than bacterial biomass, fungal activity is more likely to stimulate mineralization, although this depends on substrate quality and environmental conditions.

Soils with higher OM content are expected to have larger microbial populations and activity. Schnürer *et al.* (1985) reported significant correlations between microbial biomass and activity and SOM. Also, soils with a higher OM content, especially due to manure applications, typically have a greater N mineralization potential (Sharifi *et al.*, 2008b). Delin and Lindén (2002) found that N mineralization was controlled by OM and clay content. Soil OM is a heterogeneous mixture of organic compounds with variable molecular weights and physical locations in the soil (e.g., in the soil solution, adsorbed onto clay minerals, on the external or internal surfaces of aggregates). Consequently, the turnover of these molecules may range from days to millenia (Kleber, 2010). The labile fractions in soil OM containing N are of greatest relevance to this discussion since they are expected to rapidly cycle through the mineralization–immobilization reactions that control the supply of plant-available N (Duxbury *et al.*, 1991; Gregorich *et al.*, 1994; Haynes, 2005).

2.5.2. Soil physical properties

Soil physical characteristics such as texture exert a considerable influence on soil moisture content, porosity, and the habitat present for microbes responsible for N mineralization and nitrification, thus impacting soil N supply (Griffin, 2008; Subbarao *et al.*, 2006). The N mineralization rate is generally greater in sandy soils than in silt or clay soils due to better aeration and less physical protection of labile OM (Griffin, 2008; Sahrawat, 2008). However, sandy soils are more susceptible to N loss through leaching, which can affect the synchrony between N supply and crop N demand.

In this chapter, soil moisture and temperature are considered among the soil physical characteristics controlling soil N supply because these parameters affect microbial activity and biochemical processes in the soil. Soil moisture and temperature also influence the solubility and movement of plant-available N in the soil solution to plant roots, and the potential N loss pathways such as leaching and denitrification. [Kolberg *et al.* \(1999\)](#) stated that changes in both soil moisture and temperature have a greater effect on N mineralization than their individual additive effects alone. While the response of N mineralization to soil moisture varies with temperature, the spatial variability in soil water content is expected to be greater than the variability in soil temperature during the period of greatest crop N demand ([Drury *et al.*, 2003](#)). [Whalen *et al.* \(2001\)](#) aerobically incubated clay-loam soils collected from long-term manured plots in Alberta, Canada for 20 weeks under four different combinations of soil temperature (10 and 20 °C) and moisture (50% and 75% of field capacity (FC)) and reported higher microbial activity and greater N mineralization in hotter, wetter soils than colder, drier soils. Although mineralization did not double in response to a 10 °C increase in temperature (Q_{10}), soils incubated at 50% of FC exhibited greater Q_{10} values than soils incubated at 75% of FC. This suggests that the temperature influence on N mineralization is greater in oxic soils than those approaching anoxic conditions.

Temperature directly controls N mineralization by affecting biochemical processes and, indirectly, by affecting oxygen (O_2) consumption by microorganisms and the aerobic volume of the soil ([Sierra, 1997](#)). Mineralization rates increased linearly in five Queensland soils as temperature increased to 40 °C ([Campbell *et al.*, 1981](#)). [Griffin and Honeycutt \(2000\)](#) incubated various types of livestock manure for 112 days at 10, 17, and 24 °C and reported that the rate of nitrification increased linearly with increasing temperature. [Stanford *et al.* \(1973\)](#) noted that while ammonification continues above 35 °C, nitrification ceases at 45 °C. Temperatures between 25 and 35 °C are regarded as optimal for aerobic microorganisms ([Whalen and Sampedro, 2010](#)), yet microbial activity and organic residue decomposition continue when temperatures fall below freezing ([Breland, 1994](#)).

Soil moisture content between 50% and 80% FC is regarded as optimal for N mineralization ([Whalen and Sampedro, 2010](#)). Soil moisture content regulates O_2 diffusion for maximum aerobic microbial activity and enhances microbial mobility and diffusion of soluble substrates to microbes ([Agehara and Warncke, 2005](#)). In dry soils, reduced O_2 and substrate diffusion coupled with reduced microbial mobility and growth decreases microbial activity and N mineralization. In very wet soils, reduced activity and mobility of aerobic microbes is attributed to O_2 deprivation resulting from slow diffusion ([Paul *et al.*, 2003](#)). Nitrogen mineralization rate generally increases with soil moisture up to 60% water-field pore space ([De Neve and Hofman, 2002](#)). [Agehara and Warncke \(2005\)](#) reported net mineralized

N values from SOM of 6.9, 9.9, and 11.3 mg N kg⁻¹ at 50%, 70%, and 90% water-holding capacity, respectively after 12 weeks of incubation.

While many studies are done under constant temperature and moisture regimes, fluctuating soil temperature and moisture conditions are more realistic. Haynes (1986) suggested that fluctuating temperatures may inhibit microbial activity; however, Sierra (2002) reported similar microbial activity under fluctuating temperature as under constant temperature, when the same average temperature conditions were considered. Greater N mineralization in two fluctuating temperature patterns (FTP; FTP1: 8 h at 20 °C, 8 h at 30 °C, and 8 h at 40 °C; FTP2: 12 h at 20 °C and 12 h at 40 °C) than at a constant of 30 °C in an Oxisol was reported by Sierra (2002), while Stanford *et al.* (1975) found no effect on N mineralization of fluctuating temperatures between 5 and 35 °C in three soils incubated for 52 days. Miller *et al.* (2005) found greater net mineralization and nitrification in chaparral soils with repeated rewetting. However, Mikha *et al.* (2005) reported reduced microbial activity and cumulative N mineralization when soil was repeatedly rewetted, although a flush of mineralized C was observed after each rewetting period. Soil rewetting often causes a flush of N mineralization as microorganisms and other food-web organisms regain activity (Griffin, 2008), but the results of Mikha *et al.* (2005) suggested that rapid N immobilization occurred in response to the increase in easily accessible C after rewetting due to greater microbial activity and/or rapid growth in microbial biomass. As the soil was kept structurally intact throughout their experiment, most of the native SOM was protected from microorganisms.

2.5.3. Soil biotic properties

Interactions between soil animals and microorganisms influence the soil N supply. Grazing of soil microorganisms by soil animals such as protozoa and nematodes liberates plant-available N because the contents of the lysed microbial cells are not completely ingested or assimilated by soil animals. Grazing activities of protozoa and nematodes are influenced by soil moisture and temperature since these organisms live and move through water films (Savin *et al.*, 2001). About 30% of annual net N mineralization in soils is attributed to the activity of grazing animals (Myrold, 2005). Earthworms affect soil N supply through their feeding and casting activities, which forms middens, burrows, and casts. Subler and Kirsch (1998) reported higher concentrations of NH₄-N and dissolved organic N in middens than in bulk soil due to accelerated decomposition and mineralization. Savin *et al.* (2004) noted that earthworm burrowing accelerates N mineralization by changing the soil pore structure, resulting in increased aeration and water infiltration.

Interactions of populations within groups of soil organisms also affect soil N supply. Postma-Blaauw *et al.* (2005) showed that a contramensal

interaction between two nematode species (*Bursilla monhystra* and *Plectus parvus*) increased bacterial biomass and N mineralization in a humid podzol. In pots with different earthworms, Postma–Blaauw *et al.* (2006) showed more immobilization of mineral N into bacterial biomass with *Lumbricus rubellus* and *Lumbricus terrestris* together and *L. rubellus*—*Apporrectodea caliginosa tuberculata* mixture. However, the interaction between *A. caliginosa tuberculata* and *L. terrestris* increased bacterial growth rate and mineralization of soil C.

Biotic interactions in the rhizosphere have a profound effect on soil N supply. Higher microbial biomass and activity in the rhizosphere than the bulk soil is a well known phenomenon attributed to root-derived organic compounds known as root exudates or rhizodeposits (Mahmood *et al.*, 2005; Nannipieri *et al.*, 2008). Chantigny *et al.* (1996) studied the effect of different plant species (faba beans, alfalfa, timothy, bromegrass, reed canarygrass, and wheat) on microbial biomass in two contrasting soils and found that the differences in microbial biomass C (MBC) were significantly related to rhizodeposit C concentration under each plant species. Some studies have related increased microbial activity to increased N mineralization and potential soil N supply (Hassink *et al.*, 1993; Laungani and Knops, 2009; Puri and Ashman, 1998). Certain microbial species (e.g., *Bacillus*, *Azotobacter*, *Azospirillum*, and *Pseudomonas*) or functional groups associate preferentially with specific types of rhizodeposits and plant residues, and therefore can affect N mineralization (Singh *et al.*, 2004, 2009). Song *et al.* (2007) used 16S rRNA genes and denaturing gradient gel electrophoresis (DGGE) to analyze the AOB community composition in contrasting cropping systems. They measured significantly higher AOB in a cereal/legume intercropping system (faba beans/corn and faba beans/wheat) than in a monocropped or cereal/cereal intercropping system. Marschner *et al.* (2001) examined Eubacterial community structures in the plant rhizosphere with the same method and reported that plant species, root zone, and soil type as well as their interactions had significant effects on community structure.

2.5.4. Agricultural management

Agricultural management practices such as crop rotation, tillage, and addition of organic and inorganic amendments impact N mineralization and soil N supply through their effects on SON pool, microbial activity, and soil aggregation (Sharifi *et al.*, 2008a; Tan *et al.*, 2007). Deng and Tabatabai (2000) and Ekenler and Tabatabai (2002) reported greater N mineralization and β -glucosaminidase activity in corn, oats (*Avena sativa* L.), and alfalfa (*Medicago sativa* L.) rotations than continuous corn since crop rotations provide a greater diversity of organic materials (rhizodeposits and crop residues), thus enhancing microbial diversity, biomass, and activity. Kolberg *et al.* (1999) reported significantly higher net N mineralization in a wheat–fallow than in a wheat–corn–fallow soil; greater crop residue inputs in the wheat–corn–fallow system appeared to induce immobilization.

Soil N supply is usually enhanced when N-fixing legumes are included in the crop rotation. The response of corn to monoculture (C–C), 2-year rotation (C–S) with soybean [*Glycine max* (L.) Merr.], and 4-year rotation (C–S–W/A–A) with soybean, wheat, and alfalfa under different N input levels was evaluated by Riedell *et al.* (2009). They reported greater NO₃–N levels in soils under C–S–W/A–A across all N input levels than in the other rotations. Deep-rooted legumes like alfalfa scavenge deep residual soil N and increase N availability to subsequent shallow-rooted crops like corn and potato (Sharifi *et al.*, 2008b). Moreover, legumes are known to biological fix N₂, thereby, providing additional N to the system. Up to 150 kg N ha⁻¹ yr⁻¹ can be biologically fixed by legumes depending on year and legume species (Mueller and Thorup-Kristensen, 2001). This may account for almost 50% of total N used in agriculture (Graham, 2005). There is no doubt that legume crops can increase soil N supply, but their impacts vary widely across years and soil types, and among legume species due to differences in N₂ fixation capacity, rooting patterns, plant biomass, C/N ratio, and crop management (Chantigny *et al.*, 1996; Stark and Porter, 2005; Thorup-Kristensen *et al.*, 2003; Zebarth and Rosen, 2007). Liang *et al.* (2007a,b) reported that the addition of plant residues can significantly increase amino sugar concentration in soils, but the relative increase varied according to the plant nutrient composition and quality. The contribution of the rotation crop to soil N supply is therefore dependent on its ease of decomposition, related to the C/N ratio, NO₃–N and water-soluble compounds released from crop residue (Sharifi *et al.*, 2009c; Thorup-Kristensen *et al.*, 2003). Crop residues with a low C/N ratio are expected to decompose rapidly and cause little immobilization, thereby potentially increasing soil N supply during the early part of the growing season (Willson *et al.*, 2001). Although legumes and other crop residues with lower C/N ratios have high N mineralization potential (Sanchez *et al.*, 2004), the actual soil N supply depends on the subsequent crop species, tillage practices, soil properties, and environmental conditions (Sharifi *et al.*, 2009c; Thorup-Kristensen *et al.*, 2003).

Tillage systems affect N supply by altering soil aggregates and microbial habitats, thus affecting the access of microorganisms to labile SON compounds that can be degraded and contribute to the soil N supply. Conventional tillage systems disrupt soil structural units thereby reducing aggregation, increasing O₂ diffusion, and exposing physically protected OM to microbial attack resulting in faster turnover rates (Mikha and Rice, 2004). Also, incorporation of crop residues and animal manures in conventional tillage systems fosters better contact with the soil, resulting in faster turnover rates (Six *et al.*, 1999). Conversely, no-till systems enhance soil aggregation, and the formation and stabilization of microaggregates within macroaggregates thereby increasing the protection of OM resulting in slower turnover rates of macroaggregates (Six *et al.*, 1999). Some studies have reported greater soil microbial and enzyme activity in no-till systems due to greater soil moisture and retention of residues at the soil surface

(Acosta and Tabatabai, 2001; Deng and Tabatabai, 1996), as well as greater microbial biomass and mineralizable N (Halpern *et al.*, 2010; Sharifi *et al.*, 2008a), and greater N transformation rates (Muruganandam *et al.*, 2010). Additionally, Muruganandam *et al.* (2009) reported that no-till systems enhance N-mineralizing enzyme activities by increasing the proportion of fungi in the microbial community. In Eastern Canada, Simard *et al.* (1994) measured higher mineralizable N and a greater N mineralization rate in minimum tilled than in moldboard plowed soils.

Application of mineral and organic fertilizers can also stimulate microbial growth and activity, increase N mineralization, and soil N supply. Liang and MacKenzie (1996) reported increased microbial biomass N (MBN) due to increased mineral N fertilization in two contrasting soils in Quebec, Canada. Mikha *et al.* (2006) reported that a combination of no-tillage and manure significantly increased N mineralization and sustained soil N supply for 6 years after N application was discontinued. After 13 years of receiving solid beef (*Bos Taurus*) manure, a loam soil in Maine, USA had a significantly greater mineralizable N pool than its nonamended counterpart (Sharifi *et al.*, 2008b). Hu and Cao (2007) reported higher MBC, microbial respiration rate, and enzyme activity in compost-treated plots than inorganic fertilizer plots and untreated plots planted with winter wheat and corn. Khorsandi and Nourbakhsh (2007) found that cow manure applied at $100 \text{ mg ha}^{-1} \text{ yr}^{-1}$ for five consecutive years increased the activity of urease by 47%, L-asparaginase by 70%, L-glutaminase by 60%, and β -glucosidase by 78%.

2.6. Pathways of nitrogen loss from humid temperate soils

Nitrogen in humid temperate soils is subject to two major loss pathways: denitrification and leaching. About 40% of all soluble N entering agroecosystems in Canada is lost via leaching and denitrification (Janzen *et al.*, 2003). While NH_3 volatilization is also important, the incorporation of mineral and organic N sources has significantly reduced NH_3 volatilization from the soil (Janzen *et al.*, 2003; Rochette *et al.*, 2009). Nitrogen lost from the soil-plant system significantly reduces the plant-available N concentration, thus negatively impacting soil N supply. The form and concentration of plant-available N, soil texture and moisture, and agricultural management play major roles in defining N loss.

2.6.1. Denitrification

Denitrification is the biochemical reduction of NO_3^- or NO_2^- to gaseous N, either as molecular N or as an oxide of N under anaerobic conditions. This process involves the utilization of NO_3^- as an alternative electron acceptor for O_2 by facultative anaerobes resulting in the reduction of NO_3^- to NO_2^- , which is then subsequently reduced to NO by nitric oxide reductase, N_2O by nitrous oxide reductase and release dinitrogen gas (N_2)

as the end product (Whalen and Sampedro, 2010). Denitrification rates are highly variable (Burton and Beauchamp, 1984) and depend on environmental, soil, and agricultural management factors (Beauchamp, 1997). Dinitrogen gas represents the dominant end-product of denitrification under completely anaerobic conditions, with N_2O being dominant under partially anaerobic conditions (Drury *et al.*, 1992; Richardson *et al.*, 2009), depending on NO_3 and C availability, and soil pH (Davis *et al.*, 2008; Miller *et al.*, 2008). Moreover, the dominant form of gas emitted during denitrification is also influenced by the N_2O molar ratio ($\text{N}_2\text{O}:(\text{N}_2 + \text{N}_2\text{O})$) (Dambreville *et al.*, 2008; Mosier, 1998; Williams *et al.*, 1998) which varies in space and time (Miller *et al.*, 2008) and regulated by several environmental factors such as moisture content, temperature, redox potential, soil pH, and nitrogen oxide concentrations (Firestone and Davidson, 1989). In humid temperate regions, from 30% to 90% of annual emissions occur during the spring thaw period (Smith *et al.*, 2004; Wagner-Riddle *et al.*, 2007) due to the anoxic conditions in the soil induced by precipitation and snow melt (Teepe *et al.*, 2001) and increased diversity of active denitrifiers (Dunfield and Wagner-Riddle, 2010).

The effect of tillage on denitrification is somewhat ambiguous. Denitrification losses were found to be greater in no-till than tilled systems (Almaraz *et al.*, 2009; Baggs *et al.*, 2003; Gregorich *et al.*, 2005, 2006b, 2007, 2008; Helgason *et al.*, 2005; Tan *et al.*, 2009), which was attributed to higher soil moisture content in no-till systems. Conversely, some studies have reported greater losses in tilled soils compared to no-till (Chatskikh and Olesen, 2007; Drury *et al.*, 2006; Kaharabata *et al.*, 2003), while some reported no differences (Elmi *et al.*, 2003; Petersen *et al.*, 2008; Sey *et al.*, 2008). These ambiguous results are due to differences in annual precipitation, soil texture, and depth of tillage.

Fertilizer N and manure application contribute to denitrification by increasing $\text{NO}_3\text{-N}$ and C availability. In eastern Quebec, an increase in N fertilization of corn from 120 to 180 kg N ha⁻¹ resulted in 40–130% greater denitrification, and 50–200% higher N_2O emission (Chantigny *et al.*, 1998). Denitrification was greater in manure-treated than fertilizer N-treated plots (Chang *et al.*, 1998; Paul and Zebarth, 1997a; Rochette *et al.*, 2000), primarily due to increased C availability (Rochette *et al.*, 2000; Velthof *et al.*, 2003). Manures enhance denitrification by increasing the soil $\text{NO}_3\text{-N}$ content, supplying the soluble C that serves as the electron donor in the denitrification process, and increasing microbial activity that eventually depletes the soil O_2 concentration (Helgason *et al.*, 2005; Sey *et al.*, 2008). Interestingly, the method of placement of both mineral and organic N sources can be seen as a ‘trade off’; fertilizer incorporation reduces NH_3 volatilization, but increases the risk for denitrification in humid conditions (Janzen *et al.*, 2003; Webb *et al.*, 2010). Split applications of N fertilizer can

reduce denitrification (Burton *et al.*, 2008) depending on climatic conditions. Since denitrification is a microbial process, higher rates are expected at higher temperatures (Chirinda *et al.*, 2010; Pelletier *et al.*, 1999); however, denitrification still occurs at subzero temperatures (Phillips, 2007, 2008; Singurindy *et al.*, 2009).

2.6.2. Nitrate leaching

Nitrate leaching is the most important pathway of N loss from the soil–plant system in humid regions because of high soil moisture from annual precipitation and snowmelt, high mobility of NO_3^- , and generally higher NO_3^- than NH_4^+ concentrations in the soil solution (Myrold, 2005; Whalen and Sampedro, 2010). De Jong *et al.* (2007) developed an agro–environmental indicator to monitor potential water pollution through N leaching in Canada, and predicted an average N loss of $>20 \text{ kg N ha}^{-1}$ in the humid temperate regions. Nitrate leaching is influenced by the interaction of several factors including cropping system, tillage practices, inorganic and organic N management, soil structure, amount of water infiltration, as well as timing of the infiltration event, and poses an environmental risk since NO_3^- that is leached can eventually enter ground and surface waters leading to reduced fresh water quality and eutrophication of aquatic systems. The risk of $\text{NO}_3\text{--N}$ leaching is generally higher in shallow-rooted crops that have low N recoveries, particularly when grown on coarse-textured soils and receive large inputs of N fertilizer, for example, potato and corn. Zebarth and Rosen (2007) noted that $\text{NO}_3\text{--N}$ leaching represents the primary form of N loss in potato production. Low residual $\text{NO}_3\text{--N}$ under irrigated potatoes in eastern Canada was attributed to increased $\text{NO}_3\text{--N}$ leaching during the growing season (Bélanger *et al.*, 2003). Cumulative $\text{NO}_3\text{--N}$ loss through tile drainage from a clay–loam soil in southwestern Ontario after 3 years was 82 kg N ha^{-1} for fertilized continuous corn, 100 kg N ha^{-1} for fertilized rotation corn (corn–oat–alfalfa–alfalfa), and 70 kg N ha^{-1} for second year fertilized alfalfa (Tan *et al.*, 2002b). Soil $\text{NO}_3\text{--N}$ concentration measured at 30 cm depth in spring from 228 commercial potato fields in New Brunswick was 3–100% of soil $\text{NO}_3\text{--N}$ at harvest in the preceding fall indicating that significant leaching occurs between growing seasons (Zebarth *et al.*, 2003).

Tillage has a variable impact on $\text{NO}_3\text{--N}$ leaching. Some studies reported greater $\text{NO}_3\text{--N}$ leaching in no–till systems compared to conventional tillage systems (Patni *et al.*, 1998; Tan *et al.*, 2002a) due to formation of continuous soil macropores that increase $\text{NO}_3\text{--N}$ movement through preferential flow. Conversely, others have reported greater $\text{NO}_3\text{--N}$ leaching in conventional tillage partly due to higher rates of denitrification in no–till soils (Mkhabela *et al.*, 2008), greater infiltration in tilled soils (Drury *et al.*, 1993), or enhanced N mineralization in tilled soils (Constantin *et al.*, 2010), while some reported no differences (Fuller *et al.*, 2010; Stoddard *et al.*, 2005).

Tan *et al.* (2002a) measured 83 kg N ha⁻¹ in tile drainage water from a no-till site and 64 kg N ha⁻¹ from a conventional tillage site over a 5-year period. They attributed their results to improved wet aggregate stability, increased near-surface hydraulic conductivity, and larger earthworm populations that created continuous soil macropores in the no-till site. The effect of tillage on NO₃-N leaching will therefore differ from year to year and between spatial units.

Nitrate leaching generally increases with N fertilizer application rates. However, the timing of N application, particularly manure, coupled with the use of controlled-release N fertilizers can significantly reduce NO₃-N leaching (Fuller *et al.*, 2010; Wilson *et al.*, 2010; Ziadi *et al.*, 2011). The use of cover crops over winter can also reduce NO₃-N leaching. Hooker *et al.* (2008) reported that the use of a cover crop (mustard (*Sinapsis alba*) over winter reduced NO₃-N leaching from spring barley (*Hordeum vulgare* L.) by 38–70%. Further reductions in NO₃-N leaching losses can be achieved through improvements in N-use efficiency by synchronizing N inputs to crop N demand and efficient use of irrigation water (Zebarth *et al.*, 2009).

Although N losses through denitrification (Paul and Zebarth, 1997a) and leaching (Drury *et al.*, 1996) are problematic when they occur outside the growing season, these processes can also occur when crops are present. When soil N supply exceeds plant N demand, N mineralized during the growing season is susceptible to loss (Paul and Zebarth, 1997b). Strategies to properly account for the soil N supply, to reduce N applications in excess of plant N requirements and to synchronize N supply and plant N uptake will be critical in reversing this trend.

3. PREDICTORS OF SOIL N SUPPLY IN HUMID TEMPERATE REGIONS

Sharifi *et al.* (2007b) stated that a realistic estimate of N mineralized from SOM is essential for determining the rate of N fertilizer application required to optimize crop yield and quality, and to minimize adverse impacts of excess N on the environment. As mentioned above, soil N supply in humid regions is primarily dominated by in-season organic N mineralization due to substantial N losses over late fall and winter (Zebarth *et al.*, 2005) that vary within and between fields, as well as across years. Knowledge of the quantity of N that will potentially be available during the growing season is therefore important for improving N fertilizer management, and thus requires an estimation of the size of the labile organic N pool, which is degraded through microbial action to release plant-available N and provide the soil N supply (Curtin and Campbell, 2008).

While the size and quality of the potentially mineralizable N (N_0) (i.e., the maximum quantity of N that the soil can mineralize) is related to the soil N supply, laboratory methods assess plant-available N concentration under controlled conditions and therefore cannot predict how much will actually reach a crop due to transformations in the N cycle under specific soil and environmental conditions encountered in the field. A long-term aerobic incubation (AI) of >20 weeks under optimum temperature (35 °C) and moisture (–80 kPa) with period leaching of mineralized N was proposed by [Stanford and Smith \(1972\)](#) to estimate N_0 and its mineralization rate constant (k) using a first-order kinetic model. This method, used in its original form or modified, is generally regarded as the standard measure of N mineralization potential. However, it is time consuming and not practical for routine use. Several other laboratory methods, both biological and chemical, were developed as indices of N mineralization potential. The form of N measured by these different indices is given in [Table 1](#).

The acceptance of an N index depends on its ability to routinely predict N_0 ([Schomberg et al., 2009](#); [Sharifi et al., 2007b](#); [Wang et al., 2001](#)) as well as N supply in the field ([Griffin, 2008](#)). Examples of research using both of these approaches are summarized in [Tables 2 and 3](#), respectively. Although most SON is proteinaceous in origin ([DiCosty et al., 2003](#); [Knicker, 2000, 2004](#)), chemical methods that aim to extract these proteinaceous compounds from the soil and predict N supply across all agroecosystems are proving unsuccessful. These compounds are tightly bound to a nonextractable phase ([Knicker, 2000](#); [Knicker and Hatcher, 1997](#)) and their cycling is controlled by the interaction of site-specific factors such as soil type, moisture, and temperature, microbial diversity, and agricultural management. [Rovira et al. \(2008\)](#) noted that there is no universal pattern of decomposition, suggesting the existence of site-specific biota. These factors may explain why certain soil testing methods give reliable estimates of soil N supply in one area, but not across broad geographical regions and agricultural practices.

3.1. Biological methods

The biological methods involve soil incubation under conditions that promote N mineralization and measure inorganic N produced during a specified period of time. They are based on the fact that N transformation in soils under field conditions involves microbial-based processes. Biological methods vary by soil pretreatment (wet vs. dry, sieved vs. intact), incubation procedures (varying temperature and moisture, aerobic vs. anaerobic), incubation periods (1–40 weeks), extracting solutions (1 M KCl, 2 M KCl, 0.01 M CaCl₂), and methods of determination (colorimetric vs. steam distillation). Both NH₄-N and NO₃-N are measured during AIs, but only NH₄-N is produced under anaerobic conditions. Unfortunately,

Table 1 Nitrogen forms measured by N indices

N index	Abbreviation	Form of N measured
Biological methods		
Aerobic incubation	AI	Soluble NH ₄ and NO ₃ , N mineralized into NH ₄ and NO ₃
Anaerobic incubation	AnI	Soluble NH ₄ , N mineralized into NH ₄
Chemical methods		
UV absorbance of NaHCO ₃ extract at 205 nm	NaHCO ₃ -205	Soluble and exchangeable NH ₄ and NO ₃
UV absorbance of NaHCO ₃ extract at 260 nm	NaHCO ₃ -260	Exchangeable NH ₄ and hydrolyzable N
Hot KCl extractable N (100 °C)	H-KCl-N	Soluble and exchangeable NH ₄ , hydrolyzable N
Hot water extractable N (100 °C)	H-W-N	Soluble and exchangeable NH ₄ and NO ₃ , clay-fixed NH ₄
Direct steam distillation of NaOH extract	NaOH-DD	Exchangeable NH ₄ , amino sugars, amides, and some amino acids
Direct steam distillation of phosphate-borate buffer (pH 11.2) extract	PB-DD	Soluble and exchangeable NH ₄ , some amino acids
Gas pressure test with Ca (ClO) ₂	GPT	Soluble and exchangeable NH ₄ , hydrolyzable N
Microbial biomass C and N	MBC, MBN	Total C and N in living microbial biomass
Illinois soil N test for amino sugar N	ISNT	Amino sugar N, some amino acids, and exchangeable NH ₄
Particulate organic matter C and N	POMC, POMN	Total C and N of sand-size OM
Light fraction organic matter C and N	LFOMC, LFOMN	Total C and N of OM isolated by density fractionation
<i>In situ</i> methods		
Preplant nitrate test	PPNT	Soluble NO ₃
Presidedress nitrate test	PSNT	Soluble NO ₃
Anion and cation exchange membranes	AEM, CEM	Soluble NO ₃ and NH ₄

biological methods are unable to fully account for the frequent fluctuations in temperature and dry-wet cycles that occur under field conditions. The absence of plants in laboratory incubations is another limitation since plant

Table 2 Relationship between N indices and N_0 determined by aerobic incubation

N index ^a	Duration of incubation (days)	Coefficient of determination (R^2)	Reference
H-KCl-N	168	0.13	Curtin and Wen (1999)
H-KCl-N	168	0.26	Sharifi <i>et al.</i> (2007b)
H-KCl-N	168	0.78	Jalil <i>et al.</i> (1996)
NaHCO ₃ -205	168	0.47	Sharifi <i>et al.</i> (2007b)
NaHCO ₃ -260	168	0.74	Sharifi <i>et al.</i> (2007b)
MBC	168	0.11	Sharifi <i>et al.</i> (2007b)
POMC	168	0.47	Sharifi <i>et al.</i> (2007b)
POMC	287	0.77	Schomberg <i>et al.</i> (2009)
POMN	168	0.39	Sharifi <i>et al.</i> (2007b)
ISNT	168	0.51	Sharifi <i>et al.</i> (2007b)
NaOH-DD	168	0.61	Sharifi <i>et al.</i> (2007b)
PB-DD	168	0.11	Sharifi <i>et al.</i> (2007b)
PB-DD	168	0.73	Jalil <i>et al.</i> (1996)
GPT	287	0.83 ^b (0.57) ^c	Schomberg <i>et al.</i> (2009)

^a Abbreviations as in Table 1.

^b Correlation coefficient (r) from publication.

^c Calculated coefficient of determination (R^2).

roots alter microbial diversity, soil moisture, and exude C that stimulates the N mineralization rate (Parkin *et al.*, 2002).

Although biological methods, particularly AIs, simulate N mineralization under field conditions, they have been regarded as time consuming, expensive, and not practical for routine use (Sharifi *et al.*, 2007b). Moreover, the absence of a standardized procedure makes it difficult to compare the correlation between this and other methods.

3.1.1. Aerobic incubation

A standard long-term incubation method was first proposed by Stanford and Smith (1972) involving soil incubation for >20 weeks under optimum temperature (35 °C) and moisture (−80 kPa) with periodical leaching of mineralized N with 0.01 M CaCl₂. The potentially mineralizable N (N_0) and its mineralization rate constant (k) were assessed using a first-order kinetic model. Several studies (Campbell *et al.*, 1984, 1988; Carpenter-Boggs *et al.*, 2000) showed that N_0 or k could be adjusted to provide a better estimate of N supply with respect to fluctuations in soil temperature and moisture that occur under field conditions. In a 40-week AI at 35 °C with organic-waste amended soils, Griffin and Laine (1983) reported that yield and N uptake of corn correlated well with the $N_0 \times k$ interaction, but N_0 alone proved to be a very poor predictor of yield or N uptake. Carpenter-Boggs *et al.* (2000)

Table 3 Relationship between N indices and N supply under field conditions

N index ^a	Field assessment	Coefficient of determination (R ²)	Reference
PPNT	Potato relative yield	0.58 ^b (0.34) ^c	Sharifi <i>et al.</i> (2007a)
PPNT	N supply to corn ^d	0.75 (0.56)	Hong <i>et al.</i> (1990)
PPNT	N supply ^e	0.64	Sharifi <i>et al.</i> (2009c)
PSNT	N supply to corn	0.58	Fox <i>et al.</i> (1993)
H-KCl-N	Oats N uptake	0.79	Smith and Li (1993)
H-KCl-N	Potato relative yield ^f	0.09 (<0.01)	Sharifi <i>et al.</i> (2007a)
NaHCO ₃ -260	N supply to corn	0.86	Fox and Piekielek (1978b)
NaHCO ₃ -260	N supply to corn	0.41 (0.17)	Hong <i>et al.</i> (1990)
NaHCO ₃ -205	Potato relative yield	0.48 (0.23)	Sharifi <i>et al.</i> , 2007a
NaHCO ₃ -205	N supply	0.40	Sharifi <i>et al.</i> (2009c)
NaHCO ₃ -205	Corn yield ^g	0.83, 0.31	Nyiraneza <i>et al.</i> (2009)
MBN	Potato relative yield	0.19 (0.04)	Sharifi <i>et al.</i> (2007a)
POMC	Potato relative yield in one season ^h	0.12 (0.35)	Sharifi <i>et al.</i> (2007a)
POMN	Potato relative yield in one season	0.30 (0.55)	Sharifi <i>et al.</i> (2007a)
ISNT	EONR for Corn	0.87, 0.78	Williams <i>et al.</i> (2007b)
PB-DD	N supply to Corn	0.25 (0.1)	Hong <i>et al.</i> (1990)
AEM	Corn N uptake	0.52	Ziadi <i>et al.</i> (2006)
AEM	Canola N uptake	0.57 ⁱ , 0.86 ^j	Qian and Schoenau (1995)
AEM	Corn N uptake ^g	0.91, 0.65	Nyiraneza <i>et al.</i> (2009)
NIRS	N supply to corn	0.49	Fox <i>et al.</i> (1993)

^a Abbreviations as in Table 1.

^b Correlation coefficient (*r*) from publication.

^c Calculated coefficient of determination (R²).

^d Corn N uptake—0.75 (starter fertilizer N).

^e Potato plant N uptake measured at vine desiccation plus soil mineral N at 0–30 cm at harvest.

^f Mean of two growing seasons (*n* = 40).

^g Two growing seasons (2005 and 2006, respectively).

^h One growing season (*n* = 19).

ⁱ Thirty soils used.

^j Twenty-three soils used.

incubated soils for 184 days in a variable-temperature incubator designed to mimic field soil temperatures, and reported a strong correlation ($r = 0.89$) between corn N uptake (no fertilizer N applied) and total N mineralized. The values of N_0 and k vary depending on temperature, moisture content, and incubation duration (Wang *et al.*, 2003). Therefore, to obtain an N_0

value that is unequivocally indicative of soil N mineralization capacity, Wang *et al.* (2003) suggested that the incubation should be conducted at standard temperature (35 °C) and moisture conditions (55–65% waterholding capacity or –30 to –10 kPa), and a standard mineralization rate constant ($k = 0.054 \text{ week}^{-1}$) should be used for all soils. The standard k minimizes the effect of incubation duration from 20 to 40 weeks on N_0 (Wang *et al.*, 2003). Short-term AI has been recommended as an alternative to long-term incubation, which eliminates the need for periodic leaching. In spite of inconsistent results, AI is more likely to simulate field conditions than other methods.

3.1.2. Anaerobic incubation

Short-term anaerobic incubation (ANI) is a rapid method to quantify the $\text{NH}_4\text{-N}$ released under anoxic conditions in a soil–water slurry incubated for 7 or 14 days under anaerobic conditions (Curtin and Campbell, 2008; Schomberg *et al.*, 2009). In contrast to AI, higher temperatures that lead to more rapid mineralization can be used, more N is usually mineralized, and there is no need to optimize moisture conditions. Nevertheless, differences in sample pretreatment that affect the N mineralized and the N mineralized by AI and ANI are not always related (Curtin and Campbell, 2008). Robinson (1967) correlated N mineralized from AI and ANI with soil organic C, Kjeldahl-N in the soil, leaf N concentration, and crop yield and concluded that AI values were more satisfactory than the ANI values in determining potentially available mineral N. Gianello and Bremner (1986a) reported correlation coefficients (r) of 0.85–0.96 between NH_4 released during a 7-day ANI and net N mineralized during AI at varying temperatures (30 and 35 °C) for 2 and 12 weeks, respectively.

3.2. Chemical extraction methods

Several chemical extraction methods (Table 1) were developed to estimate potentially available N and can be used as predictors of N supply. As noted by Griffin (2008), these methods are based on the hypothesis that a specific extractant releases N from a pool of similar decomposability but generally do not require that the N pool released by extraction is chemically homogeneous or biologically meaningful. They can be more rapid and precise than biological procedures (Curtin and Campbell, 2008), and produce an index of the contribution of soil N mineralization to soil N supply (Sharifi *et al.*, 2007a). However, they do not account for the effects of environmental conditions or take into account losses of soil mineral N during the growing season due to leaching and denitrification (Sharifi *et al.*, 2007a), and are not capable of simulating the microbial-mediated release of mineral N that occurs in incubated soil (Curtin and Campbell, 2008). The lack of

consistent and strong correlative relationships across climatic conditions, soils, and management scenarios has hindered widespread adoption of these methods. The AI procedure of [Stanford and Smith \(1972\)](#), in its original form or modified, is generally used as the reference for these methods.

3.2.1. Ultra violet absorbance of NaHCO_3 -extractable N

The use of 0.01 M NaHCO_3 as a predictor of N supply was proposed by [Maclean \(1964\)](#). It is well known that organic N compounds accumulate in highly acidic soils, and that mineralization is accelerated by raising the soil pH. On this basis, it was assumed that a relationship exists between solubility and availability of N compounds to plants, and that extraction with a mild alkaline solution should simulate conditions produced by liming to near neutrality ([Maclean, 1964](#)). This method was modified by [Fox and Piekielek \(1978b\)](#) by using UV absorbance at 260 nm to measure extractable N in the extracts. Absorbance at 205 nm measures both organic and mineral N forms in the NaHCO_3 extract ([Fox and Piekielek, 1978b](#); [Sharifi *et al.*, 2007b](#)), while absorbance at 260 nm measures OM content ([Fox and Piekielek, 1978a](#)). [Sharifi *et al.* \(2009c\)](#) showed that agricultural management effects, such as crop rotation are detected by absorbance at 205 nm but not at 260 nm. They explained that, in the short-term, crop rotation increases the proportion of labile organic N (referred to as “soil mineralizable N”) in their study, but the SON content did not change. Several studies ([Fox and Piekielek, 1978b](#); [Hong *et al.*, 1990](#); [Sharifi *et al.*, 2007a,b, 2009c](#)) have reported strong correlations between soil $\text{NO}_3\text{-N}$ concentration and absorbance at 205 nm than at 260 nm. [Serna and Pomares \(1992\)](#) reported that absorbance at 205 and 260 nm were correlated with N mineralization potential (16-week AI), however, correlations were stronger for absorbance at 260 nm ($R^2 = 0.70$) than at 205 nm ($R^2 = 0.60$) in light to medium soil texture. However, [Serna and Pomares \(1992\)](#) found that N uptake by corn grown in a growth chamber was better correlated to absorbance at 205 nm than at 260 nm ($R^2 = 0.74$ and 0.30, respectively). This illustrates a discrepancy between N mineralization potential and the N supply to crops, as detected by absorbance measurements. While it might suggest that plants access a different pool of SON than microorganisms, it could also reflect the fact that correlations do not distinguish between associative and cause-and-effect relationships. In the study by [Serna and Pomares \(1992\)](#), correlation coefficients between N mineralization potential and absorbance at 205 and 260 nm were higher in sandy than loamy or clay soils due to the tendency of clay soils to form microaggregates and to protect OM.

3.2.2. Hot KCl extractable N

[Gianello and Bremner \(1986b\)](#) proposed that the $\text{NH}_4\text{-N}$ concentration of extracts from soil shaken with 2 M KCl at 100 °C for 4 h and corrected for the initial NH_4 concentration was an index of N_0 . Hot KCl-N (H-KCl-N)

includes soluble and exchangeable forms of $\text{NH}_4\text{-N}$, the water-soluble fraction of organic N, and N compounds released from lysed microbial cells (Sharifi *et al.*, 2007b, 2008b). Curtin and Wen (1999) found that H-KCl-N (corrected for initial NH_4) was not a good predictor of the cumulative amount of N mineralized after 24-week incubation at 35 °C ($R^2 = 0.25$) or of N_0 ($R^2 = 0.13$), but was a good predictor of N mineralized in the first 2 weeks of incubation ($R^2 = 0.69$). They suggested that hot KCl was selective for a water-soluble fraction of OM. However, when total NH_4 extracted in hot KCl was used (uncorrected for initial NH_4), the relationship with N_0 improved considerably ($R^2 = 0.55$). The reverse was reported by Sharifi *et al.* (2007b) in that H-KCl-N uncorrected for initial NH_4 was better related to N_0 than when corrected for initial NH_4 ($R^2 = 0.46$ and 0.26 , respectively). Although the soils from both studies were chosen to represent various management regimes (different crops, crop rotations, tillage practices, and fertilizer treatments) and climatic zones (semi-arid to humid), soils from the Sharifi *et al.* (2007b) study had a smaller range and hence a lower mean organic C and N than those from the Curtin and Wen (1999) study. Another difference was that Sharifi *et al.* (2007b) found total organic C and total organic N highly correlated with N_0 ($R^2 = 0.60$ and 0.67 , respectively), while Curtin and Wen (1999) found a low correlation between total N and N_0 ($R^2 = 0.13$). Soils used by Curtin and Wen (1999) also had higher clay contents, which can protect labile organic N fractions through surface adsorption and reduce the mineralization rate and mineralization potential. In addition, Curtin and Wen (1999) indicated that H-KCl-N was a good predictor of N_0 in soils where k (mineralization rate constant) does not vary greatly, so this test may not be appropriate for heavily manured soils. Hong *et al.* (1990) found that H-KCl-N was not a good predictor of N supply in Pennsylvania soils, many of which were well drained and heavily manured. The chemical nature of organic N hydrolyzed in hot KCl is still unknown.

3.2.3. Hot water extractable N

Hot water extractable N as an index of N_0 and predictor of N supply was proposed by Curtin *et al.* (2006). Nitrogen extracted by hot water consists of organic N (80%), and the remainder is $\text{NH}_4\text{-N}$ generated by hydrolysis of heat-labile organic N. This method measures both organic N and $\text{NH}_4\text{-N}$, whereas only $\text{NH}_4\text{-N}$ is measured in the hot KCl method. Ammonium N released by hot water at 80 °C was closely related to hot KCl $\text{NH}_4\text{-N}$ ($R^2 = 0.92$), though somewhat more $\text{NH}_4\text{-N}$ was extracted in hot KCl (Curtin *et al.*, 2006). A study examining changes in hot water extracts of two contrasting soils (alkaline fluvisol and acid cambisol) during incubation found that humic materials produced by microbial growth and death were extracted in hot water (Redl *et al.*, 1990). These results suggest that the N released by hot water (80 °C) is mainly of microbial origin. However,

Chantigny *et al.* (2010) disputed the notion that hot water preferentially extracts N-rich microbial tissues and further suggested that $\text{NH}_4\text{-N}$ released in hot water above 50 °C included clay-fixed NH_4 .

3.2.4. Direct steam distillation with sodium hydroxide extract or phosphate–borate buffer extract (pH 11.2)

The $\text{NH}_4\text{-N}$ liberated by direct distillation of soil with alkaline reagents such as sodium hydroxide (NaOH; Sharifi *et al.*, 2007b) or phosphate–borate buffer (PB; Gianello and Bremner, 1988; Hong *et al.*, 1990) were used as indices of mineralizable N. Direct distillation with NaOH was suggested by Sharifi *et al.* (2007b, 2009b), a modification of the procedure of Stanford (1978). According to Schomberg *et al.* (2009), NaOH-DD measures the most chemically resistant but hydrolyzable N pool and likely includes N from $\text{NH}_4\text{-N}$, amino sugars, amides, and certain amino acids (Sharifi *et al.*, 2007b, 2008b). A moderate relationship ($R^2 = 0.55$) was reported by Schomberg *et al.* (2009) between NaOH-DD and N mineralized after 41 weeks.

Curtin and Wen (1999) found the PB-DD was poorly related to N_0 ($R^2 = 0.09$), but was better related to N mineralized after the first 2 weeks of incubation ($R^2 = 0.64$). Good relationships between PB-DD and N_0 were reported by Jalil *et al.* (1996) ($R^2 = 0.73$) and Schomberg *et al.* (2009) ($R^2 = 0.54$). Curtin and Wen (1999) suggested that the PB solution extracts labile organic N adsorbed to mineral surfaces as well as more soluble organic N, while Gianello and Bremner (1986a, 1988) suggested that it measures a combination of $\text{NH}_4\text{-N}$ and some amino acids. Sharifi *et al.* (2008a) reported that NaOH-DD extracted a greater portion of SON than PB-DD because NaOH-DD hydrolyzed organic N adsorbed to mineral surfaces and this alkaline solution (pH 14) extracted more soluble organic N than the PB (pH 11.2).

The NaOH-DD method is sensitive to management-induced changes such as tillage and crop rotation (Sharifi *et al.*, 2008a, 2009c). Sharifi *et al.* (2007b) compared NaOH-DD and PB-DD using contrasting soils from a wide range of climatic zones and management histories, and reported a better relationship for NaOH-DD than PB-DD with N mineralized after 24-week AI ($R^2 = 0.61$ and 0.11, respectively). Hong *et al.* (1990) examined the ability of PB-DD to predict the N supply to maize in Pennsylvania soils over 3 years and concluded that PB-DD was not a good predictor of soil N supply ($r = 0.25$). Conversely, Vanotti *et al.* (1995) showed that PB-DD was a good indicator of soil N supply under field conditions in Wisconsin ($r = 0.76$ and 0.80 for corn grain yield and N uptake, respectively) and cumulative N mineralized after 4 weeks ($r = 0.79$). Jalil *et al.* (1996) reported that PB-DD was effective in detecting the effect of crop rotation on soil N supply.

3.2.5. Gas pressure test

Gas pressure is generated when a strong oxidizing reagent such as $\text{Ca}(\text{ClO})_2$ oxidizes NH_4 to N_2 gas and C to CO_2 in a closed vessel. This method was originally developed by Chescheir *et al.* (1985) to measure the NH_4 content of livestock manures and subsequently adopted by Picone *et al.* (2002) as a rapid (25 min) gas pressure test (GPT) to estimate mineralizable N and MBC in soils in Georgia. They reported strong correlation between the GPT and N mineralized in 24 days and soil MBC ($r = 0.77$ and 0.90 , respectively). In mineral soils of North Carolina, Williams *et al.* (2007b) found significant relationships between the GPT and economically optimum N rate (EONR), and delta yield (maximum yield minus check yield) for corn ($R^2 = 0.62$ and 0.60 , respectively). However, due to the low coefficients of determination obtained, Williams *et al.* (2007b) concluded that the GPT cannot be used to make N recommendations and should not be used in soils high in OM. Schomberg *et al.* (2009) reported that the GPT was not a good predictor of potentially mineralizable N of soils from southern USA ($R^2 = 0.57$). The GPT could be tested further in humid temperate soils, but mineral N transformations during the growing season may restrict its usefulness in predicting soil N supply.

3.2.6. Microbial biomass C and N

Soil microbial biomass is a relatively small component of the SOM—the MBC comprises only 1–3% of total soil C and MBN is 5% of total soil N—but they are the most biologically active and labile C and N pools (Deng *et al.*, 2000; Smith and Paul, 1990). The chloroform fumigation–direct extraction method of Vance *et al.* (1987), further described by Voroney *et al.* (2008), is the most common method to measure MBC and MBN concentrations.

Numerous studies have related MBC or MBN concentrations to N_0 in agroecosystems with contrasting results (Deng *et al.*, 2000; Franzluebbers *et al.*, 2001; Hu and Cao, 2007; Sharifi *et al.*, 2007b; Willson *et al.*, 2001). Carter and Macleod (1987) found that MBN was closely related to N_0 ($R^2 = 0.94$) in Prince Edward Island soils, while Sharifi *et al.* (2007b) reported that MBC was poorly related to N_0 ($R^2 = 0.11$) in soils from humid and semi-arid climatic zones in Canada and the USA. However, there are few reports in the literature that relate MBC or MBN (Sharifi *et al.*, 2007a) to soil N supply in arable crop fields in humid temperate regions. Microbial biomass in humid temperate regions is subjected to frequent variations in soil water potential, temperature, and substrate availability during the growing season. In deciduous forests, Holmes and Zak (1994) reported marked variability in net N mineralization during the growing season while MBC and MBN remained relatively constant. They concluded that N availability is not controlled by large seasonal fluctuations in soil microbial biomass, but rather by changes in the turnover rate of microbial biomass such that a relatively constant pool is maintained through time. Other studies also reported that the microbial biomass remains

relatively constant throughout the year in spite of changing soil conditions and nutrient availability (Patra *et al.*, 1990; Puri and Ashman, 1998). This may suggest that microbial activity, rather than the size of the microbial biomass represents a better indicator of soil N supply (Hassink *et al.*, 1993; Puri and Ashman, 1998). However, Deng *et al.* (2000) suggested that N mineralization is predominantly controlled by biochemical processes that are independent of microbial activity (i.e., from extracellular enzymes). Nevertheless, microbial activity and/or enzyme activity alone, or in combination with microbial biomass may not be ideal indicators of soil N supply since a substrate is required, and this can be influenced by amount/quality of crop residues, soil texture, moisture, and temperature.

3.2.7. Illinois soil N test for amino sugar N

Khan *et al.* (2001) and Mulvaney *et al.* (2001) noted that an estimate of a labile or organic N fraction that becomes available to the plant through mineralization during the growing season would be the ideal soil N test. This led to the development of the Illinois soil N test (ISNT) on the assumption that the amino sugar N fraction was a good predictor of corn response to N fertilization. Khan *et al.* (2001) used the ISNT to positively identify soils that were unresponsive to fertilizer N. Mulvaney *et al.* (2001) reported that soil concentrations of amino sugar N were highly correlated with check-plot corn yield ($r = 0.79$) and fertilizer N response of corn in Illinois ($r = 0.82$). They further reported greater mineral N production in the nonresponsive soils with an accompanying reduction in amino sugar N. Also in Illinois, the ISNT was significantly related to corn N requirement and correctly identified 94% of the sites that were unresponsive to fertilizer N (Mulvaney *et al.*, 2006). The ISNT was strongly related to EONR for well and poorly drained sites in North Carolina ($R^2 = 0.87$ and 0.78 , respectively) (Williams *et al.*, 2007a). In addition, Williams *et al.* (2007b) reported a strong relationship between ISNT and EONR ($R^2 = 0.90$) in mineral soils. Sharifi *et al.* (2007b) found a good correlation ($R^2 = 0.51$) between ISNT and potentially mineralizable N (AI at 25 °C for 24 weeks) in soils from humid and semi-arid regions across Canada and north-eastern USA. Conversely, Klapwyk and Ketterings (2006) and Barker *et al.* (2006b) found that the ISNT was unable to separate responsive and nonresponsive corn sites in New York and Iowa, respectively. However, Klapwyk and Ketterings (2006) reported improved prediction when OM was considered. There were no significant relationships between ISNT and relative grain yield, grain yield response, or EONR for corn (Barker *et al.*, 2006b) in Iowa. Other studies (Laboski *et al.*, 2008; Marriott and Wander, 2006; Sharifi *et al.*, 2007a) also found that the ISNT was poorly related to labile soil N and crop response to N fertilization. Yet, it was effective in accounting for potentially available N from previously applied compost and liquid dairy manure (Klapwyk *et al.*, 2006), crop rotation (Barker *et al.*, 2006a), and tillage practices at some sites (Sharifi *et al.*, 2008a).

These inconsistent results, particularly from field evaluations, can be attributed to differences in agricultural practices (manure, crop rotation and tillage system; [Barker et al., 2006a](#); [Laboski et al., 2008](#); [Sharifi et al., 2007a, 2008a](#)), climate variability ([Barker et al., 2006b](#); [Laboski et al., 2008](#)), and differences in soil characteristics including total soil C and N. For example, at L'Acadie in Quebec, Canada, SON concentration and ISNT values varied significantly ($P < 0.05$) according to tillage system ([Sharifi et al., 2008a](#)). It is important to note that the unresponsive sites identified by the ISNT in the study by [Khan et al. \(2001\)](#) historically received manure; therefore, it can be argued that the ISNT was responding to manure application history. In the study by [Laboski et al. \(2008\)](#), the previous crop and drainage class significantly ($P < 0.05$) affected the ISNT values. The soils used by [Barker et al. \(2006b\)](#) had relatively high levels of hydrolyzable $\text{NH}_4\text{-N}$ relative to amino sugar N. Mean soil OM content in soils used by [Klapwyk and Ketterings \(2006\)](#) was twice that of soils used by [Khan et al. \(2001\)](#).

The ISNT may not be selective for amino sugar N, but most likely extracts multiple fractions of soil N ([Griffin, 2008](#); [Olk, 2008a](#)). Nevertheless, [Wang et al. \(2003\)](#) concluded that the determination of any one chemical form or combination of forms of organic N may not be sufficient in estimating N_0 if other soil properties vary widely. This is evident in the different results reported in the literature. For example, the range of OM levels in the study by [Klapwyk and Ketterings \(2006\)](#) was much larger than in the study by [Khan et al. \(2001\)](#). However, the OM range was similar for studies by [Khan et al. \(2001\)](#) and [Sharifi et al. \(2007a\)](#), but with differing crops (corn vs. potato). There are currently no reports of successful field evaluations of the ISNT with crops other than corn.

3.2.8. Light fraction organic matter carbon and nitrogen (LFOMC, LFOMN), and particulate organic matter carbon and nitrogen

Light fraction organic matter (LFOM) and particulate organic matter (POM) are mainly composed of partially decomposed plant residues together with microbial products that are not closely associated with soil minerals ([Six et al., 2002](#)). They are also referred to as physically uncomplexed OM or the intermediate pool of OM between fresh plant residues and stabilized soil organic matter ([Gregorich et al., 2006a](#)), are the major sources of C and N for microbes and represent the major pathway through which N is transformed from crop residues to the soil ([Haynes, 2005](#)). Several studies have demonstrated the response of these parameters to soil management and cropping systems, but because of their nature, they reflect short-term dynamics in the SOM pool ([Angers et al., 1993](#); [Franzluebbers and Stuedemann, 2008](#); [Franzluebbers et al., 2000](#); [Griffin and Porter, 2004](#); [Janzen et al., 1992](#); [Willson et al., 2001](#)). These fractions have a higher turnover rate than total SOM ([Chan, 1997, 2001](#)). While they may exist in the same labile N pool, they differ in characteristics and association.

POM, also referred to as macroorganic matter, or sand-size or coarse POM, is associated only with the sand-sized fraction and are separated through sieving. The LFOM, also referred to as noncombined clay fraction and mineral-free debris, may be present in all particle-size fractions and are isolated by using liquids of a specific density ($1.2\text{--}2\text{ g cm}^{-3}$) such as NaI (Gregorich *et al.*, 2006a; Gregorich and Beare, 2008). According to Gregorich *et al.* (2006a), the amount of soil C and N in POM is often greater than that in LF. In addition, LFOM is more closely related to plant residues than POM, and POM has a lower C/N ratio than LFOM. In agricultural soils, POM accounts for about 22% and 18% of soil organic C and total soil N respectively, while LFOM makes up 8% and 5%, respectively. The C/N ratio of these fractions is usually wider than that of the whole soil and therefore may immobilize mineralized N (Janzen *et al.*, 1992). A study by Compton and Boone (2002) showed that LF immobilized more ^{15}N per unit of C than POM due to a greater C/N ratio. Whalen *et al.* (2000) observed that the addition of LF to soils caused N immobilization and concluded that POM was the main source of potentially mineralizable N, whereas LF is a potential sink for mineral N.

In using soils from the different agroecological regions of Saskatchewan, Curtin and Wen (1999) found a good correlation between LFOM and potentially mineralizable N ($R^2 = 0.83$), however, it was not well related to N mineralized in the first 2 weeks of incubation. There are very few reports in the literature that relate either of these fractions to soil N supply under field conditions. Particulate organic matter C (POMC) was poorly related to total N uptake and potato relative yield across sites in USA and Canada ($r = 0.32$ and 0.35 , respectively), however, particulate organic matter nitrogen (POMN) had a higher correlation ($r = 0.41$ and 0.55 , respectively) (Sharifi *et al.*, 2007a). In a subsequent study, Sharifi *et al.* (2008b) reported good predictive potential of POMN with total N uptake and N uptake + soil mineral N ($R^2 = 0.51$ and 0.66 , respectively) after harvest in a potato production system in Maine. Due to their association with microbial biomass (Franzluebbers and Stuedemann, 2003; Janzen *et al.*, 1992), accumulation of these fractions can occur during fall and winter. Their subsequent mineralization in the spring and summer depends on moisture and temperature, but may not show a consistent pattern. Hence, a better understanding of the nutrient supply capabilities of these fractions is required.

3.3. *In situ* methods

Several *in situ* methods such as buried resin bags and microplot cylinders could be used to assess plant-available N, but this chapter will focus on ion exchange membranes buried randomly in the field with no barriers to capture $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$ from the soil solution. In addition, the preplant nitrate test (PPNT) and presidedress nitrate test (PSNT) are

regarded as *in situ* methods due to the time the soil is sampled and form of N measured ($\text{NO}_3\text{-N}$) by these methods. These *in situ* methods cannot be used to predict N supply since they represent plant-available N accumulated during a short period during the growing season.

3.3.1. Preplant nitrate test

The PPNT or spring soil nitrate test measures soil NO_3 concentration within the crop root zone. Soil $\text{NO}_3\text{-N}$ concentration at this stage represents residual soil NO_3 from the previous growing season and limited N mineralization that occurs in early spring (Zebarth *et al.*, 2009). Use of the PPNT alone is not reliable in humid regions since soil N supply is controlled by N mineralization during the growing season (Zebarth *et al.*, 2005). Bélanger *et al.* (2001) reported that the PPNT alone could not adequately predict the N requirement of potatoes in Atlantic Canada, while O'Halloran *et al.* (2004) found that the PPNT was unable to delineate management zones for barley and corn in southwestern Ontario. Conversely, Sharifi *et al.* (2008b) reported a good relationship ($R^2 = 0.58$) between PPNT and potato N uptake and concluded that the PPNT has the capacity to estimate soil N supply in the field, while Bilbao *et al.* (2004) reported that the PPNT gave an accurate estimate of optimum N rate for sugar beet in Mediterranean soils with limited leaching. The PPNT was a good predictor of soil N supply in a nonamended potato field (Sharifi *et al.*, 2007a). However, Sharifi *et al.* (2007a) noted that the PPNT was problematic as a predictive test in humid regions because soil $\text{NO}_3\text{-N}$ concentrations can change rapidly during spring, making the values sensitive to sampling date, and therefore, may be more applicable in arid and subhumid environments. McTaggart and Smith (1993) attempted to estimate N_0 using the PPNT and concluded that PPNT was not a practical method for determining spring fertilizer applications in eastern Scotland. These findings suggest that the PPNT is unable to fully and consistently account for N mineralization during the growing season, and may be incapable of detecting management-induced changes on potentially mineralizable N in humid temperate regions.

3.3.2. Presidedress nitrate test

The PSNT was developed to improve N fertilization for corn in humid regions (Magdoff *et al.*, 1984), and is based on the assumption that the amount of $\text{NO}_3\text{-N}$ present just before rapid plant N uptake is directly related to the soil N supply during the growing season. Soil $\text{NO}_3\text{-N}$ concentration represents the residual $\text{NO}_3\text{-N}$ from the previous growing season, N mineralized from manure application and/or fertilizer N application in early spring, and N mineralized from SOM up to sampling time (generally June–July). Nitrogen mineralized from SOM prior to sampling may be as much as 60% of apparent growing season soil N mineralization (Zebarth *et al.*, 2001). Schepers and Meisinger (1994) stated that the late spring increase in $\text{NO}_3\text{-N}$ from *in situ*

mineralization depends on recent additions of crop residues or manure. The PSNT was developed for corn as a sidedress fertilizer management tool, and requires careful sampling within a short period of time and rapid analysis. Soil $\text{NO}_3\text{-N}$ concentration between 21 and 25 mg kg^{-1} indicates sufficient N for corn to attain optimum yield (Fox *et al.*, 1989), and lower values suggest that sidedress N fertilizer application is required. However, frequent rainfall, large spatial variability and rapid changes in soil $\text{NO}_3\text{-N}$ concentrations in humid regions (Ma *et al.*, 2007), coupled with sampling time, labor requirement, and cost have made it difficult to rely on the PSNT. Hence, it is better suited for identifying sites for which a yield response to additional N at sidedress is unlikely to occur, than for predicting the amount of fertilizer N required during the entire growing season (Klausner *et al.*, 1993). Heckman *et al.* (2002) indicated that the PSNT is most useful in cropping situations where significant amounts of mineral N may be expected in the soil prior to the period of major N uptake by the crop, especially in situations where manure was applied, or where forage crops were included in the rotation. The PSNT has been used in other crops such as sweet corn (Heckman *et al.*, 1995; Ma *et al.*, 2007), fall-grown cabbage (*Brassica oleraceae* L.; Heckman *et al.*, 2002), lettuce (*Lactuca sativa* L.), and celery (*Apium graveolens* L.; Hartz *et al.*, 2000). Essentially, determination of critical soil $\text{NO}_3\text{-N}$ concentration at the critical crop growth stage is vital for the success of the PSNT.

3.3.3. Anion and cation exchange membranes

In situ ion exchange membranes measure soil N supply at certain points in time since they are exposed to the same environmental conditions and edaphic factors that affect N mineralization and subsequent N uptake by plant roots (Qian *et al.*, 1992; Qian and Schoenau, 2002; Subler *et al.*, 1995; Ziadi *et al.*, 2006). However, this method cannot be used to predict N supply and is labor-intensive, especially due to the large number of samples required to identify treatment differences due to spatial variability in N mineralization.

According to Sharifi *et al.* (2009a), ion exchange membranes act as sinks similar to plant roots, and provide an index of actual N supply over time in soils. This index can be affected by duration of the membrane burial in the soil, competing sinks (microbes and roots), waterlogging, and landscape variations (Qian and Schoenau, 2002; Ziadi *et al.*, 1999). Ziadi *et al.* (2000) found a significant relationship between $\text{NO}_3\text{-N}$ sorbed on anion exchange membrane (AEM) and EONR ($R^2 = 0.45$) as well as relative forage yield in Quebec ($R^2 = 0.60$). A strong relationship between cumulative N uptake by forages and $\text{NO}_3\text{-N}$ sorbed on AEM over two seasons ($R^2 = 0.92$ and 0.88 in spring 1995 and 1996, respectively) from four sites in Quebec was reported by Ziadi *et al.* (1999). Ziadi *et al.* (1999) also reported that $\text{NO}_3\text{-N}$ sorbed on AEMs were significantly correlated to $\text{NO}_3\text{-N}$ extracted by water, and that forage N uptake was better related to $\text{NO}_3\text{-N}$ sorbed on AEMs than to $\text{NO}_3\text{-N}$ extracted by water. Sharifi *et al.* (2009a)

used Plant Root Simulator probes (PRSTM) that contained ion exchange membranes encapsulated in plastic probes to measure soil N supply in potato fields in Prince Edward Island and Nova Scotia. They found strong linear relationships between cumulative N supply rate at 31 days after planting measured by PRSTM and total N uptake measured at vine removal ($R^2 = 0.60$), and total N uptake plus soil mineral N in 0–30 cm at harvest ($R^2 = 0.60$). [Ziadi *et al.* \(2006\)](#) reported significant relationships between NO₃-N adsorbed on AEMs and extracted by water in forage production ($R^2 = 0.95$), extracted by 2 M KCl in corn production ($R^2 = 0.66$), cumulative N uptake by forages ($R^2 = 0.94$), and N uptake by grain corn ($R^2 = 0.52$). [Nyiraneza *et al.* \(2009\)](#) also found good relationships between NO₃-N adsorbed on AEM (2 M KCl extraction) measured over two growing seasons (2005 and 2006) and corn yield ($R^2 = 0.83$ and 0.63, respectively) and N uptake ($R^2 = 0.91$ and 0.65, respectively). Using soils from Saskatchewan in two growth chamber experiments (30 soils in experiment 1 and 24 soils in experiment 2) and ANI, [Qian and Schoenau \(2005\)](#) reported significant relationships between NH₄-N sorbed on cation exchange membranes (CEMs) and N uptake by canola ($R^2 = 0.67$ and 0.73 in experiments 1 and 2, respectively).

[Zebarth *et al.* \(2009\)](#) stated that ion exchange membranes are most valuable when a rapidly growing crop depletes the soil mineral N sufficiently that direct measurement of soil mineral N during the growing season does not provide a meaningful estimate of plant-available N. Ion exchange membranes are superior to traditional *in situ* methods since they allow for minimal soil disturbance, ensure complete contact with the soil, measure mineral N fluxes that occur with changing environmental conditions over time, and reflect the cumulative effects of soil chemical, physical, and biological properties, as well as agricultural practices such as tillage, crop rotation, and manure application. However, like most other methods, there are limitations. Although a large number of membranes can be placed in the field, the actual size of the membranes and hence sampling area is relatively small. Also, the flux cannot be converted to relevant values for developing fertilizer recommendations. Moreover, the soil N supply varies from year to year, so the flux measurements from ion exchange membranes are not predictive.

3.4. Optical method

3.4.1. Near-infrared reflectance spectroscopy

The methods described previously are based on wet chemistry, sometimes require hazardous chemicals, and are expensive and time consuming. Near-infrared reflectance spectroscopy (NIRS) is an alternative method that is rapid, cost-effective, nondestructive, and environmentally safe ([Dunn *et al.*, 2002](#); [Galvez-Sola *et al.*, 2009](#); [Nduwamungu *et al.*, 2009b](#)). Near-infrared reflectance mainly measures overtones and combination bands of vibrations

of O–H, N–H, and C–H bonds found in organic constituents and in soil mineral particles (Sorensen and Dalsgaard, 2005). Therefore, direct quantitative estimates of NIR–spectra are impossible. Data are interpreted with multivariate statistics such as partial least squares regression, multiple linear regression, and principal components analysis (Börjesson *et al.*, 1999; Chang *et al.*, 2001). Coefficient of determination (R^2), ratio of performance deviation (RPD), ratio of error range (RER), and regression coefficient (b) were used by Nduwamungu *et al.* (2009b) to compare NIRS predictions with soil attributes in Eastern Canada. Calibration libraries will need to be validated against reference data on the same samples, before predicting concentrations of constituents in unknown samples (Malley *et al.*, 2002). The standard error in reference data (SER) should be reported, along with the standard error of prediction (SEP; Nduwamungu *et al.*, 2009a).

Nduwamungu *et al.* (2009a) discussed the application of NIRS for assessing primary and secondary soil properties. Primary properties that have a theoretical basis for NIRS predictions include particle size, moisture content, organic C, and total N, while secondary properties such as EC, CEC, pH, potentially mineralizable N, and microbial biomass have no theoretical basis but are correlated to certain primary properties. Moreover, portable NIRS have potential for “on site” determination of soil properties such as OM ($R^2 > 0.85$), CEC ($R^2 > 0.85$), and moisture content ($R^2 > 0.94$) (Sudduth and Hummel, 1993a,b). Vasques *et al.* (2009) used NIRS to predict total SOC and SOC fractions in soils in Florida and reported coefficients of determination (R^2) ranging from 0.42 to 0.70. Reeves and Van Kessel (2000) reported that NIRS successfully determined moisture ($R^2 = 0.95$), total C ($R^2 = 0.95$), total N ($R^2 = 0.96$), and $\text{NH}_3\text{-N}$ ($R^2 = 0.97$) concentrations in dairy manures. Nduwamungu *et al.* (2009b) accurately predicted soil texture, CEC, total C, total N, and potentially mineralizable N in noncalcareous soils in Quebec, Canada, and NIRS successfully predicted soil nutrient concentrations and properties in other soils (Chang *et al.*, 2001; Cozzolino and Morón, 2003; Malley *et al.*, 1999; Martin *et al.*, 2002). The NIRS was used to predict N supply in corn ($R^2 = 0.49$; Fox *et al.*, 1993) and winter wheat ($R^2 = 0.81$; Börjesson *et al.*, 1999).

The use of NIRS for predicting soil N supply is promising, particularly with homogeneous soil samples, but requires further development of standard reference methods (biological or chemical) and a large spectral library.

4. CONCLUSIONS AND FUTURE WORK

Numerous methods that measure N mineralization potential have been tested for their ability to predict soil N supply, but none has proven robust enough for broad acceptance. An ideal method would predict the

crop N uptake during the growing season, be inexpensive and rapid, and respond to N dynamics as influenced soil properties, agricultural practices, and environmental conditions. Soil N supply therefore appears to be system-specific, that is, it differs according to crop rotation, tillage system, organic amendment, soil properties, and most importantly, environmental conditions. This is evident since in many instances, an N test that has proven successful in one system failed to accurately predict the soil N supply in another. Therefore, accurate determination of N availability during a growing season in humid environments requires a holistic approach that considers a specific system. The following represents possible alternatives to current methodologies.

4.1. Combination of N indices plus modeling with weather data

A single N index is insufficient to predict the contribution of N mineralization to soil N supply for agroecosystems at a regional scale. Hence, a combination of N indices that are best suited for the particular system in conjunction with weather forecasts may be more accurate. Schomberg *et al.* (2009) showed that combining indices that represent an available substrate (e.g., total N) and microbial biomass (e.g., flush of CO₂ during 3 days; Franzluebbbers *et al.*, 2000) can improve predictions of N_0 but the choice of indices depends on clay mineralogy and OM content. Kay *et al.* (2006) showed that variation in N supply can be explained by the interaction between the temporal variation in weather and the spatial variation in soil characteristics. Moreover, Kay *et al.* (2006) reported that early season rainfall exerted a greater influence on N supply than rainfall later in the season, so N fertilizer recommendations could be adjusted based on early season weather conditions. Dessureault-Romppe *et al.* (2010) integrated soil and climatic parameters to explain 63% of the variation in N_0 across Canada by combining a soil texture, a SOM, and a climatic parameter. However, the influence of agricultural practices such as tillage, crop rotation, and organic amendment application which are known to influence the soil mineralizable N pool were not considered. Therefore, more research is needed to evaluate the potential of combined soil N supply predictors in association with early season weather data across different agricultural practices.

4.2. Identification and quantification a specific N fraction that contributes to N supply

A good soil N test for temperate humid regions would measure plant-available N plus the organic N pool that makes a dominant contribution to N supply. Identifying the labile N forms in soils that supply the plant-available N pool and quantifying their turnover rate, as a function of soil

moisture and temperature, soil properties, and agricultural practices, will enhance the development and adoption of the appropriate test. For example, the development and use of the ISNT for amino sugar N by Khan *et al.* (2001) and Mulvaney *et al.* (2001) was based on this concept and identified sites that were unresponsive to N fertilization. Although the ISNT may not be selective for amino sugar N (Griffin, 2008; Olk, 2008a), it is important to note that the unresponsive sites identified by the ISNT historically received manure (Khan *et al.*, 2001); therefore, it can be argued that the ISNT was responding to manure application history. Subsequent evaluations of the ISNT across nonmanured, crop rotation or tillage sites were not very promising. This suggests that the dominant pool varies according to the production system as well as the soil properties and climatic condition.

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