Evaluation of Soil Tests for Predicting Nitrogen Mineralization in Temperate Grassland Soils

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Improving nitrogen (N) fertilizer-use efficiency (N_fUE) in temperate grassland soils is important for agronomic and environmental sustainability. Nitrogen supplied from soil N reserves through N mineralization processes need to be accounted for when prescribing additional N fertilizer applications. Therefore a rapid and reliable soil N testing procedure for predicting mineralizable N (MN) needs to be identified for temperate grassland soils. In this study, the MN across 35 mineral grassland soils (depth 0–10 cm) from Ireland, with varying physiochemical properties, was investigated using a 7-d anaerobic incubation (AI-7) as a reference method. The soil N pools measured using seven relatively rapid chemical N indices were also examined and compared with the AI-7 values. The Illinois Soil N Test (ISNT), total N (TN), and total C (TC) correlated strongly with MN, ($r > 0.81$) across these 35 soils. There was no distinct improvement when soils were grouped according to soil textural class. Although TN and TC displayed strong relationships with MN, these N indices may be less sensitive to short-term changes due to soil management practices and climates and consequently may be less suitable as an indicator. The ISNT was the best predicator of MN, explaining 69% of the variability in MN, suggesting that it was measuring a similar labile soil organic N pool to that released by AI-7. The ISNT shows potential to replace AI-7 as a more rapid and reliable soil N test for routine analysis across temperate soil types, however, further field based studies are required.

Abbreviations: AI-7, 7-d anaerobic incubation; Cold_KCl$_{NH4}$, KCl extractable NH_4–N; Cold_KCl$_{NO3}$, KCl extractable NO_3+NO_2–N; DCR, digital color reader; Fl_CO$_2$, flush of carbon dioxide in 24 h; Hot_KCl$_{NH4}$, heated (100°C) KCl extractable NH_4–N; Hyd_N, hydrolyzable N determined by subtracting Cold_KCl$_{NH4}$ from Hot_KCl$_{NH4}$; ISNT, Illinois soil nitrogen test; k, mineralization rate constant; KMnO$_4$_oxd_N, potassium permanganate oxidation measured as NH_4–N; LOI, loss on ignition; N_fUE, nitrogen fertilizer-use efficiency; MN, mineralizable N measured as net increase mg NH_4–N kg^{-1} after 7-d anaerobic incubation; N_o, potentially mineralizable N; TC; total soil carbon; TN, total soil nitrogen; UV, ultraviolet; UV_260, ultraviolet absorption of KCl extract at 260 nm wavelength; UV_210, ultraviolet absorption of KCl extract at 210 nm wavelength; SMB, soil microbial biomass; SOM, soil organic matter.

Soil N supplied for plant growth through mineralization of soil organic matter (SOM) is often ignored in intensive agricultural systems (Griffin, 2008). In grassland systems, the emphasis and dependency is placed on N inputs alone to meet the grass N requirements over a long growing season where N is continuously removed during either grazing or cutting (Whitehead, 1995). Within the Republic of Ireland, the input of fertilizer N to meet demand is adjusted to account for stocking rate (live units ha^{-1}) in grazed grassland situations and limited by the European Union Nitrates Directive (European Community, 1991), which has set a compulsory program of on-farm measures aimed at improving national and European water quality (OJEC, 2000). Ninety percent of Irish utilizable ag-

ricultural land is grassland, mainly supporting dairy and mixed livestock enterprises (Wall et al., 2011) with outdoor grazing averaging 239 d of the year (Laepple et al., 2012), and during the winter housing period conserved grass silage is the main diet. Current measures of N availability test for soil mineral N levels, however this is unreliable due to the temporal nature of mineral N pools under humid and high rainfall climates (Keeney, 1982). Under the temperate climates of Ireland the ability to accurately predict availability of soil N for uptake by grass would facilitate improved N_fUE and help to reduce environmental losses of N. The concerns attributed to unrecovered N fertilizer by the plant include the increased emissions of nitrous oxide (N_2O) into the atmosphere, and increased risk of leaching of nitrate (NO_3^-) to groundwater (Stark and Richards, 2008).

Development and practical application of methods for measuring soil N supply through mineralization has taken place over a number of decades. However, the various soil N testing methods developed have had mixed levels of success (Keeney, 1982; Bundy and Meisinger, 1994; Griffin, 2008; Ros et al., 2011b). Although, some research has included isolated forms of physical uncomplexed organic matter such as light fraction and particulate organic matter (Gregorich et al., 2006; Kader et al., 2010); the majority of soil N tests fall into two main categories; biological or chemical.

Biological N techniques include short- and long-term soil incubations that measure relative production of inorganic N produced by the mineralization process over a defined period (Fox and Piekielek, 1984; Sharifi et al., 2007b). Incubated conditions create a soil environment that promotes biological activity, resulting in the mineralization of labile organic N pools (Dahnke and Vasey, 1973). According to Ros et al. (2011a), the measure of potentially mineralizable N (N_o), which uses a long-term incubation in combination with curve-fitting, reflects the total quantity of soil organic N that can be mineralized. In comparison, short-term incubations (aerobic and anaerobic) measure the actual quantity of organic N that mineralizes N over a specified period of time under controlled conditions (Ros et al., 2011a). In this current study the latter measure is referred to as MN.

Originally employed by Stanford and Smith (1972), long-term aerobic incubation (initially 30-wk) is one of the most widely used reference tests. In this method the conditions within the soil are standardized (35°C with daily or two to three weekly 5-min periods of aeration) to measure the pool of N_o in the form of nitrate (NO_3–N) and ammonium (NH_4–N; Campbell et al., 1993; Schomberg et al., 2009; Ros et al., 2011a). In addition the rate constant (k) of N mineralization is determined using a first-order kinetic model.

Anaerobic and aerobic incubations are noted as reference methods for estimating MN from SOM and have been used in a majority of studies evaluating the effectiveness of various soil N supply indices (Keeney, 1982). However, variances in the long-term aerobic incubations concerning the estimation of N_o and k have resulted in criticism based on the consistency of the kinetics models used (Griffin, 2008), for example, zero-order (linear) vs. first-order (nonlinear, which includes single and double exponential models; Beauchamp et al., 1986; Deans et al., 1986). Concern has also been expressed towards the sensitivity of the estimated values (N_o and k) based on the duration of incubation (<100 d; Griffin, 2008) and sample pretreatment (e.g., dried vs. frozen vs. field moist; Beauchamp et al., 1986). Similar issues have also been raised with short-term aerobic incubations (e.g., 28 vs. 56 d) and the lack of a standardized incubation temperature used among studies (Curtin and Campbell, 2006). Short-term anaerobic incubation, as proposed by Waring and Bremner (1964), has been justified in many studies as a suitable alternative approach to short- and long-term aerobic incubations (Gianello and Bremner, 1986; Soon et al., 2007; Schomberg et al., 2009; Mariano et al., 2013). This method creates warm anoxic conditions in the soil, (i.e., by water- logging), thus killing facultative aerobic microbes and adding to the biomass (Griffin, 2008), which anaerobic microbes mineralize though the processes of aminization (conversion of proteins to amine and urea residues) and ammonification (N residues to NH_4–N; Rajendra and Power, 1997). The result is only NH_4–N release over a short period of time, typically 7 or 14 d (Griffin, 2008), as the nitrification process is inhibited (van Eekeren et al., 2010). The application of higher temperatures (40°C) allows greater quantities of N release over short-term aerobic incubations (Keeney, 1982).

Reliable as these biological incubations are, they are rarely used for routine soil N testing as they are too time-consuming and impractical for high throughput analysis in soil laboratories (Keeney, 1982; Bundy and Meisinger, 1994; Wilson et al., 1994; Ros et al., 2011b; St Luce et al., 2011). Chemical methods have been proposed as alternatives, where studies have evaluated their effectiveness to extract the mineralizable soil N fraction that correlates to the standard biological measures (Selles et al., 1999; Bushong et al., 2007; Sharifi et al., 2007a; Schomberg et al., 2009). Numerous chemical N indices have been assessed worldwide with varying levels of success (Bushong et al., 2007; Griffin, 2008; Ros et al., 2011b).

Extraction of soluble and labile forms of NH_4–N using heated potassium chloride (KCl) was initially proposed by Øien and Selmer-Olsen (1980) and Whitehead (1981) as a suitable index of plant available N. Gianello and Bremner (1986; KCl 100°C instead of 80°C) reported strong correlations ($r = 0.95$) with biological incubation methods when they accounted for the initial NH_4–N levels; whereby the quantities of NH_4–N extracted at room temperature (Cold_KCl$_{NH4}$) are subtracted from the NH_4–N quantities that are extracted when heated (Hot_KCl$_{NH4}$), and the difference defined by Schomberg et al. (2009) as being hydrolyzable N (Hyd_N). However in studies by Jalil et al. (1996) and Curtin and Wen (1999), the relationships between heated KCl and N_o measured by a 24-wk aerobic incubation were found to be better ($r^2 = 0.78$ and 0.55, respectively), than when the initial NH_4–N was uncorrected.

The measurement of N by ultraviolet (UV) absorbance at 260 nm of filtered 0.01 M sodium bicarbonate ($NaHCO_3$) soil extract (Maclean, 1964) was initially proposed by Fox and Piekielek (1978). They suggested that inorganic N was in relatively constant supply from SOM, and under the correct UV wavelength SOM concentrations in a soil extract could be measured. Fox and Piekielek (1978) compared UV absorbance measurements to the N uptake of six corn sites in Pennsylvania over 2 yr and found a strong correlation ($r = 0.87$). Many studies have continued to adapt the UV wavelength measurements and soil extract for this method (Hong et al., 1990; Yasrebi et al., 2004; Sharifi et al., 2007b). Intead of $NaHCO_3$, Bushong et al. (2007) used a filtered 1 M KCl soil extract read at 260 nm and found a moderate relationship with N mineralized during a 14-d anaerobic incubation ($r^2 = 0.56$). They reported a poorer relationship ($r^2 = 0.31$) with the MN when the extract was read at 210 nm following the removal of NO_3–N according to Norman and Stucki (1981). The absorbance of the soil extract within the UV 260-nm range relates to the extractable labile organic N forms of N, but the spectra of NO_2 and NO_3 cannot be absorbed at this range. However, both labile and mineral N forms can be measured in the lower UV ranges, such as 210 nm (Norman and Stucki, 1981; Norman et al., 1985; Nyiraneza et al., 2012). To compare the capacity of both UV ranges to measure the labile organic N within the soil extract, $NO_2 + NO_3$–N is removed (Bushong et al., 2007).

Stanford and Smith (1978) evaluated the effectiveness of different acidic potassium permanganate ($KMnO_4$) extraction techniques, which they showed to have strong relationships with biological incubations predicting N_o in soils across various studies (approximate $r^2 = 0.80$). Stanford and Smith (1978) suggested that acidic $KMnO_4$ oxidization of soil organic N is a good predictor of N availability. Based on Kjeldahl method, in which the supernatant of an acidified $KMnO_4$ extract was centrifuged and distilled with sodium hydroxide (NaOH), Wilson et al. (1994) reported significantly strong relationships with an AI-7 ($r^2 = 0.90$ to 0.95) using a 3:1 and 4:1 soil extract ratio, respectively. Using a similar procedure, Bushong et al. (2007) found that acidic $KMnO_4$ oxidation was the strongest predictor of MN after a 14-d anaerobic incubation ($r^2 = 0.58$) when a range of chemical soil N indices were assessed across 50 soils from South-Central and Midwestern United States.

Some studies recognize the flush of carbon dioxide (CO_2), indicating the size and activity of the soil microbial biomass (SMB) present, as a good biochemical indicator of MN in soils (Franzluebbers et al., 1996; Haney et al., 2001; Schomberg et al., 2009). Based on the observations from these studies, practical short-term procedures for measuring the flush of CO_2 evolved from rewetting of dried soil have been developed. Using soil samples taken over two separate years, Haney et al. (2001) reported that the flush of CO_2 after 1 d significantly ($p < 0.01$) explained 78 to 93% of the variability in N mineralized in a 24-d incubation. Similar results were reported by Schomberg et al. (2009) in which the flush of CO_2 after 3 d explained 64% of the

variability in N_o, determined using a fixed k. For more routine soil analysis, this method has been further developed by Haney et al. (2008), where the alkali traps and acid titration were replaced with sensitive pH gel paddles with built in color indicators for CO_2 levels.

The amino sugar fraction of soil organic N was identified by Mulvaney and Khan (2001) as a labile source of nitrogenous metabolite released during N mineralization and shown to be a good predictor of corn response to N fertilizer. Based on these finding, Khan et al. (2001) developed the Illinois Soil Nitrogen Test (ISNT), which replaces the lengthy acid hydrolysis method for determining amino sugars with direct alkaline hydrolysis. Subsequent to this: various studies have reported strong correlations between ISNT-N and biologically based N indices N_o (Sharifi et al., 2007a; Nyiraneza et al., 2012) and MN (Bushong et al., 2008). Klapwyk and Ketterings (2006) found the combination of SOM with ISNT-N improved the prediction of corn sites that were responsive to N fertilizer in New York. Some studies have reported that ISNT-N has been unsuccessful in predicting field sites that are non-responsive to N fertilizer applications (Barker et al., 2006; Osterhaus et al., 2008; Spargo et al., 2009), whereas other studies reported positive correlations with plant N uptake, especially when soils were grouped based on similar soil classifications, drainage class (Williams et al., 2007a, 2007b) and sampling depth (Roberts et al., 2011).

Few studies have investigated the capabilities of chemical N indices to predict MN in temperate grassland soils with high and relatively stable SOM content (Brogan, 1966). Large ranges in grass N uptake have been reported from such soils without any N fertilizer additions across 16 United Kingdom (UK; 40–150 kg N ha^{-1} yr^{-1}), 21 Dutch (43–233 kg N ha^{-1} yr^{-1}) and 15 Irish (74–212 kg N ha^{-1} yr^{-1}) soils (Hopkins et al., 1990; Hassink, 1995; Humphreys, 2007). These values were attributed to mineralized N from labile fractions of SOM. However, in many temperate regions, for example, in Ireland, the potential supply of N from grassland soils is not accounted for when making N fertilizer recommendations. With such a wide range of available N that could be supplied from the mineralization process of native soil N reserves; there is a risk that the existing application rates of N fertilizer could be over or under supplying the needs of grassland soils. Therefore, this highlights the need to develop a reliable and quick N test that can predict soil N supply from N mineralization in temperate grassland systems, to accurately prescribe N fertilizer rates for specific site-soil combinations.

Grouping data based on climatic zone and/or common soil properties such as organic matter content and texture has been applied in many studies (Bushong et al., 2007; Sharifi et al., 2007a; Nyiraneza et al., 2012), to use site homogeneity as the differential to improve the prediction power of MN. However, Sharifi et al. (2007a) also noted that these referred groupings gave no improvement in the relationship of N indices (including ISNT-N) with N_o using a 24-wk aerobic incubation. When Bushong et al. (2007) and Nyiraneza et al. (2012) grouped

their data based on textural difference, the outcomes differed. Bushong et al. (2007) found that the prediction of MN using a 14-d anaerobic incubation improved using ISNT-N when 16 silt loam sites were isolated from the original 50 evaluated (r^2 = 0.45 to 0.71) and similar improvements were also reported for other N indices UV_260, UV_210, and $KMnO_4$_oxd_N. When Nyiraneza et al. (2012) grouped data into fine and coarse-medium textured soils there was no significant improvement from N indices such as ISNT-N in N_o (using a 24-wk aerobic incubation) from the original 54 soil data set.

Biological N indices, such as the AI-7 test are recognized as being suitable indictors of a soil's MN potential (Gianello and Bremner, 1986; Soon et al., 2007; Schomberg et al., 2009; van Eekeren et al., 2010); therefore AI-7 is a suitable reference to evaluate less time-consuming chemical N indices against. Given the lack of knowledge regarding the effectiveness of many of the aforementioned chemical indices in predicting soil N availability in temperate grassland soils, the objectives of this study were to: (i) determine the range of mineralizable N in temperate grassland soils varying in morphology and texture and (ii) evaluate a suite of chemical N indices for predicting MN measured by AI-7, and (iii) evaluate the use of different soil properties for improving the relationship between chemical N indices and MN measured by AI-7.

MATERIALS AND METHODS
Soil Sampling and Preparation

Thirty-five soil samples were collected from a range of characteristic permanent grassland sites across the Island of Ireland in 2010, represented in Table 1. Approximately 5 kg of soil was randomly collected to a depth of 10-cm (as per the standard agronomic soil sampling depth for grasslands in the Republic of Ireland) across a representative sampling area of 0.405 ha. Each of the fresh composite samples was passed through a 6.3-mm sieve to remove root mass and large rock materials, with a 0.25-kg subsample taken and stored at <4°C. Each soil sample was analyzed for soil moisture content (van Reeuwijk, 2002). The remaining soil was dried at 40°C, passed through a 2-mm sieve and stored at room temperature for future analysis.

Soils were analyzed for inorganic phosphorus (P) (Morgan, 1941), potassium (K), and magnesium (Mg) using the method of Byrne (1979) and biocarbonate-extractable inorganic P based on the Olsen et al. (1954) method using a soil to Olsen reagent of 1:20. Ethylenediaminetetraacetic acid (EDTA) extractable (Byrne, 1979) copper (Cu), zinc (Zn), and easy reducible manganese (Mn) were determined using a Varian VISTA Inductively coupled plasma–optical emission spectroscopy (ICP–OES; Varian, Palo Alto, CA). Sulphur (S) was extracted with 0.01 M calcium phosphate monobasic monohydrate [Ca $(H_2PO_4)_2$ H_2O] using the auto-analyzer method (Sinclair, 1973) and extractable sodium (Na) using method 67 by Ministry of Agriculture, Fishers, and Food (1986), while concentrations were determined using a Varian Liberty Plasma 96- ICP (Varian, Palo Alto, CA). Soil pH (1:2) was measured in water (Byrne, 1979) using a Mettler Toledo electrode pH meter (Mettler-Toledo, Zurich, Switzerland). Soil organic matter (SOM) was determined by loss-on-ignition (LOI) at 500°C for 16 h (Storer, 1984). Soil texture was determined by particle-size analysis using the pipette method (Avery and Bascomb, 1974) and USDA classification of sand, silt, and clay (Brady and Weil, 2002). Total N (TN) and total carbon (TC) for soil was analyzed by dry combustion using a CN LECO FP 2000 analyzer (LECO Corp., St. Joseph, MI).

Table 1. Soil location and classification of temperate soils used to evaluate N indices.

ID	Site	County	Soil classification†	Location
1	Brookesbrough	Fermanagh	Typic Epiaquept	54°20′52.398′N, 7°25′09.743′W
2	Sixmilecross	Tyrone	Lithic Haplohumod	54°32′06.868′N, 7°13′49.559′W
3	Sixmilecross	Tyrone	Humic Epiaqualf	54°31′27.896′N, 7°13′29.718′W
4	Sixmilecorss	Tyrone	Lithic Haplahumod	54°32′14.238′N, 7°13′23.566′W
5	Kircubbin	Down	Humic Eutrudept	54°28′07.164′N, 5°31′33.373′W
6	Kircubbin	Down	Humic Eutrudept	54°28′04.825′N, 5°31′45.568′W
7	Sixmilecross	Tyrone	Lithic Haplahumod	54°32′08.699′N, 7°13′27.276′W
8	Brookesbrough	Fermanagh	Typic Epiaquept	54°205′1.531′N, 7°25′04.329′W
9	Ballymagorry	Tyrone	Humic Dystrudept	54°52′32.958′N, 7°23′20.007′W
10	Ballymagorry	Tyrone	Typic Eutrudept	54°51′58.359′N, 7°23′47.446′W
11	Kildalton	Kilkenny	Typic Epiaquept	52°21′24.566′N, 7°18′06.891′W
12	Kildalton	Kilkenny	Typic Eutrudept	52°20′54.855′N, 7°18′29.768′W
13	Kildalton	Kilkenny	Typic Hapludalf	52°21′33.524′N, 7°19′01.924′W
14	Ballycanew	Wexford	Typic Epiaqualf	52°37′24.082′N, 6° 18′54.274′W
15	Ballycanew	Wexford	Typic Eutrudept	52°35′30.598′N, 6°22′54.038′W
16	Johnstown Castle	Wexford	Aquic Hapludalf	52°17′13.419′N, 6° 30′42.380′W
17	Johnstown Castle	Wexford	Typic Dystrudept	52°17′46.107′N, 6° 30′22.526′W
18	Castledrockrell	Wexford	Typic Eutrudept	52°33′57.159′N, 6° 36′56.038′W
19	Castledrockrell	Wexford	Typic Endoaquept	52°35′27.527′N, 6°37′08.144′W
20	Screen	Wexford	Orthic Haplorthod	52°25′31.784′N, 6°25′24.165′W
21	Screen	Wexford	Orthic Haplorthod	52°25′48.092′N, 6°25′40.895′W
22	Timoleague	Cork	Entic Haplorthod	51°37′00.048′N, 8°46′58.008′W
23	Timoleague	Cork	Entic Haplorthod	51°37′18.569′N, 8°47′58.591′W
24	Dunleer	Louth	Typic Hapludalf	53°49′51.006′N, 6°25′01.002′W
25	Dunleer	Louth	Typic Hapludalf	53°49′44.004′N, 6°26′02.004′W
26	Kilworth	Cork	Typic Hapludalf	52°09′43.197′N, 8°13′59.209′W
27	Kilworth	Cork	Typic Hapludalf	52°10′15.187′N, 8° 14′21.408′W
28	Kilworth	Cork	Typic Hapludalf	52°10′10.741′N, 8° 14′47.285′W
29	Coroduff	Monaghan	Humic Eutrudept	54°01′44.216′N, 6°52′27.253′W
30	Sreenty	Monaghan	Humic Eutrudept	54°01′52.098′N, 6°51′44.244′W
31	Cregduff	Mayo	Typic Eutrudept	53°37′02.886′N, 9°09′45.522′W
32	Cregduff	Mayo	Typic Eutrudept	53°35′56.004′N, 9°06′00.018′W
33	Grange	Meath	Typic Endoaquept	53°31′11.152′N, 6° 39′42.825′W
34	Grange	Meath	Typic Endoaquept	53°31′22.649′N, 6°40′07.597′W
35	Cushendall	Antrim	Typic Dystrudept	55°05′08.297′N, 6°03′54.504′W

† Soil Survey Staff (2010).

Ferrous iron (Fe^{2+}) levels were determined with tri(2′-pyridyl)-l,3,5-triazine (TPTZ) on soils stored at $<4°C$ in accordance with an adapted procedure by Krishnamurti and Huang (1990).

Biological Analysis of Soil Nitrogen Mineralization
Anaerobic Incubation

The anaerobic incubation procedure used to determine MN was based on the methods of Keeney and Bremner (1966) and Schomberg et al. (2009), in which 5 g of dried soils was waterlogged in a 16 × 150 mm (outer dimension) screw-capped test tube and incubated at 40°C for 7 d (AI-7). After incubation, soils were extracted with 25 mL of 2 M KCl, and inorganic N (NO_2–N, NO_3–N, and NH_4–N) concentrations were analyzed colorimetrically using an Aquakem 600A (Aquakem 600A, 01621, Vantaa, Finland). The quantity of N mineralized was calculated as the difference in NH_4–N concentrations between Day 7 and time zero extractions, with three replicate samples for each soil.

Chemical Soil Nitrogen Indices
Extractable Inorganic Nitrogen

Extractable inorganic N (NO_2+NO_3–N and NH_4–N) was determined using a method adapted from Keeney and Nelson (1982) and Mulvaney (1996). One hundred milliliters of 2 M KCl was added to 50 g of fresh soil in a 250-mL container, which was then shaken on an orbital shaker for 1 h, and the extract filtered through Whatman no. 40 filter paper. Concentrations of extractable NO_2–N, NO_3–N (Cold_KCl$_{NO3}$), and NH_4–N (Cold_KCl$_{NH4}$) were determined by colorimetric analysis using a continuous flow analyzer SKALAR SAN++ (Skalar Analytical B.V, Breda, The Netherlands).

Hot Potassium Chloride Extractable NH_4–N

Hot extractable NH_4–N (Hot_KCl$_{NH4}$) was determined following the procedure of Schomberg et al. (2009). Twenty milliliters of 2 M KCl was added to 3 g of fresh soil ($<4°C$) in a 20-mL centrifuge tube and incubated in a 100°C ($±1°C$) water bath for 4 h. Extraction, filtration, and analysis for Hot_KCl$_{NH4}$ was as described for cold inorganic N. Hydrolyzable N (Hyd_N) was determined by subtracting Cold_KCl$_{NH4}$ from Hot_KCl$_{NH4}$.

Mild Acidic Potassium Permanganate Oxidation

The mild acidic $KMnO_4$ oxidation of N (KMnO$_4$_oxd_N) followed the method of Bushong et al. (2007). Thirty milliliters of 0.05 M $KMnO_4$ + 0.5 M sulfuric acid (H_2SO_4) was added to a 50-mL centrifuge tube containing 10 g of dried and sieved (<2 mm) soil. The mixture was shaken on an orbital shaker for 1 h, centrifuged for 10 min at $850 × g$, and the supernatant filtered through Whatman no. 40 paper. Ten milliliters of the extract was treated with 15 mL of 10 M sodium hydroxide (NaOH) and distilled into 5 mL of boric acid (H_3BO_3). The NH_4–N concentration was determined colorimetrically using an Aquakem 600A (Aquakem 600A, 01621, Vantaa, Finland).

Ultraviolet Absorption at 260 and 210 nm

Ultraviolet (UV) absorption at 210 and 260 nm was performed according to the method of Bushong et al. (2007). Twenty-five milliliters of 1 M KCl was added to 5 g of dried soil, shaken for 1 h, filtered through Whatman no. 40 filter paper and read at 260 nm (UV_260) on a CARY 50 Conc. UV-VIS Spectrophotometer (Varian, Palo Alto, CA). Nitrate in the filtrate was reduced to non-absorbing NH_4–N by adding 0.3 g of Devarda's alloy and 0.5 mL of 20% (v/v) H_2SO_4 into a glass test tube that contained the 5 mL of 1 M KCl extract and then heated in an oven at 60°C for 35 min, filtered as previously described and diluted 9:1 (water/filtrate) with deionized water and read using the same spectrophotometer at 210 nm (UV_210).

Respiration Flush of Carbon Dioxide

The relative flush of CO_2–C (Fl_CO$_2$) was estimated using the Solvita Haney-Brinton test kit for C and N mineralization potential as developed by Haney et al. (2008) and Haney and Haney (2010). For three replicate soil samples, 40 g of dried soil was weighed into a 50-mL plastic beaker, which was modified with holes at the bottom and covered with a Whatman no.GF/D (4.25-cm) glass micro-fibre filter. The plastic beaker was then placed into a 250-mL glass jar. Avoiding this beaker of soil, 25 mL of deionized water was added into the glass jar. Immediately a color gel indicator probe (bright blue in color) was read for initial CO_2 levels using a Solvita digital color reader (DCR; Woods End Laboratories, Mt. Vernon, ME; resolution of 0.5 mg kg^{-1}) and then carefully placed alongside the beaker of soil into the jar facing outwards and enclosed with a screw tight lid. The start time was recorded, and the jar placed into an incubator set at 23.5°C [midway between the recommend 22–25°C (Woods End Laboratories, 2011)] for 24 h. After the elapsed time, the jars were reopened and read for CO_2–C in mg kg^{-1} using the DCR.

Illinois Soil Nitrogen Test

The ISNT by Khan et al. (2001) of direct diffusion of soils was used to estimate the amount of amino-sugars plus NH_4–N in the soil. For three replicate soil samples, 1 g of dried and sieved (<2 mm) soil was treated with 10 mL of 2 M NaOH in a modified 473-mL (1 pint) wide-mouth Mason jar. A 60-mm Petri dish containing 5 mL of 2% boric acid was suspended from a modified lid of the jar which was sealed to be air-tight. Following modifications suggested by Klapwyk and Ketterings (2005), the sealed jar was placed onto an enclosed hot plate at 50°C ($±1°C$) for 5 h. The concentration of ISNT-N liberated by the NaOH and captured as NH_4–N by the boric acid was quantified by colorimetric analysis with an Aquakem 600A (Aquakem 600A, 01621, Vantaa, Finland).

Statistical Analysis

All statistical analyses were performed using SAS JMP 9.0 (SAS Institute, Cary, NC). Summary statistics and one way analysis of variance were performed on all replicated data. Linear correlations among all N indices were examined using

Pearson correlation coefficients. The relationship between the soil properties (Table 2) and chemical N indices (Table 3) with MN (determined using AI-7) was examined using stepwise regression analysis of the linear and quadratic terms of each soil property and chemical N index. A significance level of $\alpha = 0.05$ was applied in all regression analysis.

RESULTS AND DISCUSSION
Variation of Soil Properties and Soil Nitrogen Indices

The physical and chemical properties of 35 soils are presented in Table 2. The soils were classified into four textural classes; loamy sand ($n = 2$), sandy loam ($n = 5$), loam ($n = 24$), and clay loam ($n = 4$). The 35 mineral soils used in this study represent a wide range of soil pH, SOM, TN, and TC (Table 3), bio-available concentrations of primary (Morgan's P, Olsen's P, K, Mg, and S) and secondary nutrients (micronutrients; Mn, Cu, and Zn; Table 2).

The MN (measured using AI-7) ranged from 92 to 403 mg kg^{-1} NH$_4$–N (Table 3). These values were greater than reported in other studies by Keeney and Bremner (1966; 4–112 mg kg^{-1}), Gianello and Bremner (1986; 22–161 mg kg^{-1}), Soon et al. (2007; 2.9–80.8 mg kg^{-1}) and Schomberg et al. (2009; 4–115 mg kg^{-1}). However, the soils investigated in these previous studies were predominately from arable farming systems, typically sampled at the 0- to 15-cm depth and had humid climates but colder winters than in Ireland. These previous studies do not represent long-term grassland soils under temperate climates, where high biomass production levels over long growing seasons is common (Laepple et al., 2012), and where SOM concentrations usually increase over time (Whitehead, 1995). The high MN concentrations found in the

Table 2. Summary of the textural content and available nutrients for 35 temperate grassland soils used to evaluate mineralizable N.

| ID | Soil texture | | | pH | P† | Olsen P‡ | K | Mg | Cu | Zn | Mn | S | Na | Fe$_2$$^+$ |
	Sand	Silt	Clay											
	g kg-1				mg L^{-1}									
1	557	322	121	5.4	13.9	67.8	121.2	199.8	5.6	6.3	20.8	11.1	27.5	0.7
2	266	453	281	5.2	6.7	36.0	155.2	144.4	6.6	3.8	91.2	11.8	32.0	2.0
3	292	429	279	5.1	4.0	21.7	139.0	126.5	3.9	4.8	111.8	11.6	35.5	1.2
4	298	384	319	5.3	8.9	58.5	145.9	174.6	6.9	5.9	181.9	9.4	46.5	1.2
5	436	349	214	5.5	10.3	27.0	99.0	313.0	12.0	6.1	140.4	14.8	47.5	1.1
6	503	296	200	5.5	18.7	51.3	292.6	354.9	15.0	7.7	167.8	15.6	58.0	3.1
7	372	376	252	6.1	10.8	38.9	96.5	122.7	8.9	3.3	171.9	14.4	27.5	0.9
8	682	206	111	5.1	15.2	87.3	215.9	172.0	5.9	7.0	196.8	13.4	38.5	2.0
9	688	231	81	5.5	3.8	18.1	107.1	94.0	8.2	2.7	43.7	12.1	29.5	1.2
10	570	315	115	5.8	7.5	36.9	218.3	184.3	8.2	3.7	256.0	10.2	33.0	0.8
11	354	402	244	5.8	4.7	13.4	41.8	194.0	5.7	3.4	117.6	9.5	25.5	3.0
12	355	412	233	5.4	1.8	12.3	43.7	124.5	3.8	3.2	439.8	6.2	19.5	0.7
13	362	396	242	6.3	12.7	39.1	153.7	117.6	5.0	3.3	423.6	9.9	22.5	1.3
14	319	374	307	6.2	6.9	15.0	83.8	522.2	7.3	4.6	94.1	6.9	32.5	1.4
15	381	398	221	5.5	2.3	16.7	82.9	114.4	12.5	6.6	404.8	6.4	23.5	0.7
16	446	387	167	6.0	4.5	28.9	123.7	188.1	4.3	3.6	312.0	10.0	29.0	1.0
17	462	369	169	5.7	6.0	20.0	82.1	312.4	10.8	6.8	256.7	10.7	30.5	1.1
18	268	484	248	5.7	1.8	11.8	74.1	192.6	7.6	3.3	182.5	8.4	22.5	0.6
19	345	407	248	5.7	1.8	15.1	81.7	216.7	5.8	4.2	259.9	9.3	27.5	0.6
20	812	118	70	5.4	5.1	9.3	108.9	137.3	1.6	2.1	213.3	4.4	19.5	0.6
21	752	153	95	5.2	2.0	33.1	54.8	149.5	1.0	1.8	71.3	6.6	22.0	0.7
22	412	420	168	5.7	12.0	51.4	97.0	141.2	13.8	8.2	211.3	8.8	31.5	2.0
23	407	434	159	5.2	5.0	28.9	66.5	133.5	12.9	8.4	283.8	9.9	41.0	1.2
24	365	396	239	5.6	21.8	55.6	256.9	296.6	8.7	11.0	361.0	10.8	29.0	0.6
25	375	382	243	5.4	2.3	10.1	58.3	106.7	9.9	4.7	217.5	6.4	34.0	1.1
26	502	329	170	6.0	33.7	85.1	57.2	107.4	41.0	40.4	141.3	6.8	21.5	1.8
27	443	379	178	5.5	4.1	23.9	36.8	70.0	10.1	8.7	301.2	9.8	18.0	1.6
28	472	335	194	6.6	40.2	85.4	68.1	100.4	39.4	50.8	229.7	7.0	19.5	0.6
29	227	423	351	5.2	2.6	15.0	167.5	138.6	8.8	2.9	168.4	13.6	29.0	1.0
30	447	286	266	5.7	4.7	36.6	372.0	181.0	17.0	5.6	448.8	12.3	26.5	0.8
31	373	403	225	5.8	5.6	26.0	111.4	110.6	10.7	5.1	144.5	8.2	28.5	0.8
32	335	438	227	6.1	2.3	8.2	89.5	95.3	4.5	1.4	230.1	8.3	28.0	1.0
33	273	500	228	5.5	7.4	26.9	205.1	155.3	9.1	8.9	346.4	11.8	27.0	1.0
34	203	469	328	5.7	3.0	8.6	37.6	129.0	6.1	3.7	193.9	10.0	28.0	1.6
35	592	235	173	4.9	5.6	34.1	111.7	184.2	8.1	5.1	163.8	11.4	49.0	1.5

†Morgan phosphorus (P) test (Morgan, 1941) is the standard laboratory test for inorganic P in the Republic of Ireland.
‡ Olsen P test is the standard laboratory test for biocarbonate-extractable inorganic P in Northern Ireland and Great Britain.

soils in this study are typical of soils developed under permanent pasture which have higher soil C and N concentrations (Whitehead, 1995; Herlihy, 1972). In Ireland, Herlihy (1972) also found lower concentrations of MN across 19 arable soils, measured using a 14-d anaerobic incubation, which ranged from 33 to 139 mg kg^{-1} (mean, 75 mg kg^{-1}) with associated low soil organic C and N concentrations (21–44 g kg^{-1} and 2.4–4.4 g kg^{-1}, respectively).

The amounts of NO_2–N extracted from the soils were negligible, most likely due to rapid enzymatic conversion of inorganic N by the nitrification process (Norton, 2008). Nitrate-N (NO_3–N) concentrations extracted by Cold_KCl also ranged widely (1 to 77 mg kg^{-1}) across the 35 soils in this

study (Table 3). However, extractable NO_3–N was shown to be a poor indicator of MN, agreeing with Norton (2008), probably because NO_3^- ions are either available for immediate plant uptake or vulnerable to losses though leaching or denitrification. On average, this relatively transient component of soil N (Cold_KCl$_{NO3}$) accounted for 0.45% of the total N in these soils.

There was a large range in NH_4–N extracted from the 35 soils using the more rigorous Hot_KCl extraction procedure (Table 3). The Hot_KCl procedure was capable of extracting additional labile organic N (as NH_4–N) from the soil, as the mean concentration (51.1 mg kg^{-1}) was 13.9 times greater than the mean level extracted by the Cold_KCl method (3.70 mg kg^{-1}; Table 3). Therefore, in comparison

Table 3. Chemical and biological N indices for 35 temperate grassland soils.

ID	TN	TC	SOM	MN†	ISNT-N	Fl_CO$_2$	Hot_KCL$_{NH4}$	Hyd_N	Cold_KCl$_{NH4}$	Cold_KCl$_{NO3}$	KMnO$_4$_oxd_N	UV_260	UV_210
	——g kg^{-1}——				————————————————mg kg^{-1}————————————————								
1	5.0	58.5	121.5	382.6	498.6	66.0	41.0	33.6	7.4	24.6	184.1	0.481	0.238
2	7.8	100.0	201.0	385.0	592.1	69.4	88.4	85.0	3.4	17.9	121.1	0.567	0.281
3	6.8	73.3	155.0	403.3	530.3	62.1	83.7	81.3	2.4	27.0	128.8	0584	0.364
4	7.3	83.6	152.0	338.3	522.0	70.0	82.3	78.5	3.9	37.7	126.3	0.517	0.397
5	5.1	51.1	115.0	245.0	374.2	62.4	65.3	65.3	0.0	57.6	143.3	0.677	0.390
6	5.7	57.3	122.0	296.8	426.3	65.8	77.4	74.4	2.9	76.9	148.3	0.832	0.368
7	5.7	65.3	138.5	289.3	447.1	68.3	59.6	55.7	3.8	41.5	128.4	0.662	0.226
8	4.3	43.6	93.5	231.8	362.0	55.3	44.9	44.9	0.0	29.1	162.5	0.456	0.215
9	4.1	53.1	110.0	174.9	282.8	64.9	52.3	50.1	2.2	31.6	145.0	0.450	0.236
10	3.6	38.5	89.0	235.4	279.9	59.0	53.8	53.7	0.1	22.2	142.4	0.491	0.239
11	4.4	44.2	107.5	221.1	346.2	67.8	53.2	52.2	1.0	19.0	123.3	0.442	0.161
12	3.3	30.5	83.5	179.2	236.2	51.8	41.1	41.1	0.0	13.5	121.1	0.292	0.117
13	2.4	22.6	66.5	194.4	187.6	50.0	104.5	104.5	0.0	19.9	98.9	0.252	0.107
14	5.1	57.2	136.5	364.7	346.0	66.3	50.8	50.8	0.0	12.0	101.7	0.532	0.157
15	3.1	32.2	87.0	136.0	251.7	47.8	45.6	45.6	0.0	12.1	126.6	0.241	0.088
16	3.9	40.5	83.0	237.4	267.4	50.4	30.3	26.8	3.5	12.7	122.6	0.342	0.272
17	4.1	40.7	92.0	260.6	300.8	61.3	43.4	41.2	2.2	15.0	130.0	0.452	0.121
18	5.2	61.5	148.5	283.8	399.3	65.8	36.6	32.4	4.1	22.8	153.5	0.448	0.132
19	4.1	43.1	114.0	213.3	311.8	54.6	33.5	30.2	3.2	10.1	139.5	0.305	0.102
20	2.2	24.3	55.5	125.4	222.3	44.7	29.1	25.4	3.7	6.2	113.0	0.270	0.093
21	2.4	24.5	61.0	92.2	208.1	42.9	29.9	24.7	5.2	1.0	115.4	0.264	0.099
22	4.9	47.7	112.0	216.5	346.9	64.6	36.0	36.0	0.0	21.4	175.5	0.464	0.138
23	4.9	48.0	112.0	192.3	380.8	64.2	47.0	47.0	0.0	37.9	172.3	0.453	0.186
24	5.2	50.5	120.0	234.1	319.9	58.7	52.4	41.1	11.3	3.6	140.9	0.433	0.129
25	3.1	30.2	86.5	143.1	279.6	46.1	29.1	18.3	10.8	6.5	103.9	0.247	0.075
26	3.5	35.1	83.0	161.4	284.6	61.0	35.1	29.4	5.7	16.6	145.5	0.421	0.114
27	3.5	33.1	81.0	151.1	300.8	59.0	30.1	21.9	8.2	14.3	146.9	0.357	0.097
28	3.9	40.4	94.5	233.0	413.9	48.1	39.0	31.3	7.6	18.4	194.8	0.525	0.128
29	6.7	80.8	175.0	334.8	479.4	65.8	46.6	43.9	2.7	8.9	152.1	0.455	0.157
30	5.4	54.7	135.5	214.7	407.2	54.9	40.8	38.0	2.7	11.8	143.7	0.336	0.099
31	4.6	47.2	111.0	260.3	358.6	64.0	78.7	70.2	8.5	20.1	137.6	0.377	0.135
32	4.2	41.1	105.5	273.5	342.0	66.9	51.7	42.1	9.6	8.2	122.9	0.374	0.125
33	5.0	46.5	104.0	335.8	389.1	60.4	45.0	43.0	2.0	17.5	143.6	0.429	0.136
34	5.1	47.9	107.5	246.2	386.1	64.9	46.3	42.6	3.7	15.2	124.5	0.417	0.129
35	4.6	48.3	104.0	257.8	372.3	61.0	64.6	57.2	7.4	4.7	128.2	0.362	0.112
Mean	4.6	48.5	110.4	224.2	355.8	59.6	51.1	47.4	3.7	20.4	137.4	0.434	0.176
CV%	28.7	35.2	28.3	31.8	26.7	12.9	37.0	41.6	90.1	75.2	16.1	30.0	52.2

† MN, mineralizable N measured as net increase in NH_4–N kg^{-1} after 7-d anaerobic incubation; ISNT-N, Illinois Soil Nitrogen Test N; Fl_CO$_2$, flush of carbon dioxide after rewetting dried soil measured as CO_2–C in mg kg^{-1}; Hot_KCl$_{NH4}$, heated at 100°C KCl extractable NH_4–N; Hyd_N, Hydrolyzable N determined as the difference between hot and cold extractable NH_4–N; Cold_KCl$_{NH4}$, cold KCl extractable NH_4–N; Cold_KCl$_{NO3}$, cold KCl extractable NO_3+NO_2–N; KMnO$_4$_oxd_N, potassium permanganate oxidation measured as mg NH_4–N kg^{-1}; UV_260, ultraviolet absorption of KCl extract at 260 nm wavelength; UV_210, ultraviolet absorption of KCl extract at 210 nm wavelength.

there was little difference between the range in Hot_KCl_{NH4} (29–104 mg kg^{-1}) and Hyd_N (18–104 mg kg^{-1}; Table 3) for these temperate soils. Based on associated higher SOM and biomass content, concentrations of extractable NH_4–N are typically greater in grassland than in cultivated soils (Haynes, 2000). This explains why other studies using arable soils have not reported an increase of this magnitude in labile NH_4–N, when KCl soil solutions were heated for 4 h at 100°C. However, large ranges in NH_4–N concentrations extracted by Hot_KCl were reported by Jalil et al. (1996; 9.5–87.3 mg kg^{-1}) from cultivated soils of Saskatchewan, Canada, and by Nyiraneza et al. (2012; 5–81 mg kg^{-1}) from cultivated soils of the Eastern and Western provinces of Canada.

Using the $KMnO_4\_oxd\_N$ procedure, the range of mineralized N measured as extractable NH_4–N was much narrower (99–195 mg kg^{-1}) compared with the levels obtained by other N indices (Table 3). However, $KMnO_4\_oxd\_N$ extracted greater overall quantities of NH_4–N (mean, 137 mg kg^{-1}), on average accounting for 3% of total N, when compared with the mean NH_4–N levels extracted by Hot_KCl_{NH4} and $Cold\_KCl_{NH4}$ (Table 3), which only accounted for on average 1.12 and 0.08%, respectively. Narrow ranges in concentrations using $KMnO_4\_oxd\_N$ were also reported by Wilson et al. (1994; 35 to 90 mg kg^{-1}) from silt loam soils cultivated for rice production in Southern USA. Bushong et al. (2007) also reported a narrow range in N recovery using $KMnO_4\_oxd\_N$ (48–93 mg kg^{-1}) from cultivated soils with varying textural classes and concluded that this procedure was not as sensitive as biological incubation methods for discriminating among the mineralization capacity of differing soil types.

The mean values of Fl_CO_2 after 24 h ranged from 42.9 to 69.95 mg CO_2–C kg^{-1} (2.89–4.19 Solvita digital number; Table 3). These ranges registered within the calibrated limits of 5 to 100 mg CO_2–C kg^{-1} soil (0 to 5 digital number) of the Solvita gel probe DCR (Haney and Haney, 2010). The range in Fl_CO_2 concentrations across the 35 soils is narrow in comparison with the total C concentrations of these soils (22.6–100 g kg^{-1}; Table 3), and the reported approximate ranges of 10 to 75 mg CO_2–C from 50 soils of various cropping history and soil types sampled across seven states in the USA (Haney et al., 2012). However, published results using the relatively new USDA approved Solvita Haney–Brinton test kit procedures (Haney et al., 2008) are limited, especially for studies on European soils. Therefore it is difficult to make meaningful comparisons with the ranges of CO_2–C measured from the soils in this study.

The ISNT-N ranged from 188 to 592 mg kg^{-1} (Table 3). These are comparable with the concentrations measured by Klapwyk and Ketterings (2006; 161–524 mg kg^{-1}) in sandy-silt loam soils sampled from North-Eastern USA corn sites. These sites were predominantly on dairy farms (0- to 20-cm depth) with a history of manure applications. Although the ISNT-N concentrations in these studies were similar, the SOM content of the temperate soils in this present study were greater (55.5 to 201 kg^{-1}; Table 3), than in the soils studied by Klapwyk and Ketterings (2006; 26 to 88 g kg^{-1}). This infers that the ISNT is only measuring a readily mineralizable component of SOM (i.e., amino sugars) and NH_4–N and that measuring the size of the SOM pool will be a less reliable indicator of N supply in soils. Previous land use management practices and soil depth may influence the ISNT-N concentrations of soils (Klapwak and Ketterings, 2006; Wall et al., 2010; Sharifi et al., 2011). The ISNT-N concentrations in arable soils that received little or no manure studied by Nyiraneza et al. (2012; 61–423 mg kg^{-1}) and cultivated and pasture soils with various management practices evaluated by Bushong et al. (2007; 63–368 mg kg^{-1}) were lower than the values reported by Klapwak and Ketterings (2006), as soils had received manure applications therefore producing higher ISNT-N concentrations over time (Klapwyk et al., 2006). In comparison with the other N indices shown in Table 3, ISNT-N represented on average 7.7% of total N, and MN (as measured by AI-7) on average 5.3% of total N across the soils and was higher than the other N indices measured.

Relationships between Chemical Nitrogen Indices and (Physical) Soil Properties

With the exception of $Cold\_KCl_{NH4}$, ISNT-N was the only chemical N index that was significantly correlated with all other N indices having the strongest correlation with TN, TC, SOM, Fl_CO_2, and UV_260 (r = 0.65 to 0.93, p < 0.0001; Table 4). This indicates that these soil N indices (tests) all seem to measure consistent proportions of the total N pool across these soils. However, in the case of TN, TC, and SOM indices, these may be less sensitive to changes in MN within a growing season. Wall et al. (2010) reported similar strong correlations between ISNT-N and SOM over ten arable sites; however, there was no correlation between these N indices when examined over time within each site. This suggests that the ISNT can measure shorter term changes in MN (i.e., over a growing season) but that TN, TC, and SOM cannot. The ISNT measures a specific N pool (amino sugar-N), which has been shown to be highly representative of the labile organic N pool and is potentially made available for plant uptake through N mineralization (Mengel, 1996; Roberts et al., 2009).

The Fl_CO_2 test was also highly correlated with the majority of N indices. However, there was no significant relationship observed with $KMnO_4\_oxd\_N$. The flush of CO_2 after 24 h was most strongly correlated with TN, TC, SOM, and UV_260 (r = 0.65 to 0.74, p < 0.0001; Table 4). These moderate to strong correlations indicate that the labile proportion of the total N represented by the Fl_CO_2 remained relatively constant across these soils. While the ISNT uses chemical hydrolysis to identify labile N fractions that are potentially mineralized, the Fl_CO_2 test measures CO_2–C release. This represents the labile C and N fractions mineralized by SMB, in response to substrate availability following the rewetting of dried soils (Kieft et al., 1987; Schomberg et al., 2009).

Both the UV_260 and UV_210 procedures correlated with the same N indices (TN, TC, SOM, ISNT, Fl_CO_2, Hot_

Table 4. Pearson correlation coefficients (*r*) indicting associations among laboratory indices of N availability within temperate grassland soils (*n* = 35).

N indices†	MN	TN	TC	SOM	Cold_KCl$_{NH4}$	Cold_KCl$_{N03}$	Hot_KCl$_{NH4}$	Hyd_N	Acid_oxd_N	UV_260	UV_210	Fl_CO$_2$
TN	0.819***											
TC	0.805***	0.965***										
SOM	0.781***	0.942***	0.781***									
Cold_KCl$_{NH4}$	−0.045	−0.051	−0.055	−0.048								
Cold_KCl$_{N03}$	0.278	0.372*	0.322	0.252	−0.338*							
Hot_KCl$_{NH4}$	0.487**	0.465**	0.456**	0.411*	−0.160	0.434**						
Hyd_N	0.474**	0.454**	0.447**	0.403*	−0.322	0.473**	0.986***					
Acid_oxd_N	0.127	0.198	0.158	0.146	0.034	0.298	−0.250	−0.245				
UV_260	0.620***	0.660***	0.618***	0.559**	−0.172	0.800***	0.411*	0.423*	0.338*			
UV_210	0.557**	0.611***	0.604***	0.472**	−0.263	0.756***	0.529**	0.551**	0.117	0.760***		
Fl_CO$_2$	0.686***	0.747***	0.722***	0.708***	−0.070	0.436**	0.402*	0.398*	0.205	0.651***	0.466**	
ISNT-N	0.828***	0.927***	0.910***	0.875***	0.063	0.368*	0.396*	0.369*	0.348*	0.653***	0.571**	0.672***

† MN, mineralizable N measured as net increase NH_4–N kg^{-1} after 7d anaerobic incubation; TN, total soil nitrogen; TC; total soil carbon; SOM; soil organic matter; Cold_KCl$_{NH4}$, KCl extractable NH_4–N; Cold_KCl$_{NO3}$, KCl extractable NO_3+NO_2–N; Hot_KCl$_{NH4}$, heated KCl extractable NH_4–N; Hyd_N, Hydrolyzable N determined as the difference between hot and cold extractable NH_4–N; $KMnO_4$_oxd_N, potassium permanganate oxidation measured as NH_4–N; UV_260, ultraviolet absorption of KCl extract at 260 nm wavelength; UV_210, ultraviolet absorption of KCl extract at 210 nm wavelength; Fl_CO$_2$, flush of carbon dioxide in 24 h; ISNT-N, Illinois Soil Nitrogen Test N.

* significant correlations at $p < 0.05$

** significant correlations at $p < 0.01$

*** significant correlations at $p < 0.0001$.

KCl_{NH4}, Hyd_N, and Cold_KCl$_{NO3}$), with the only exception being a weak correlations between UV_260 and $KMnO_4$_oxd_N ($r = 0.34$, $p < 0.05$; Table 4). These correlations were always significantly greater with UV_260 (Table 4). Sharifi et al. (2007a) found soil extracted with $NaHCO_3$ measured in the lower UV range (205 nm) correlated better with Cold_KCl$_{NO3}$ ($r = 0.45$, $p < 0.01$) than those read in the higher UV range (260 nm), as NO_3 should not be detectable in the higher UV range (Norman and Stucki, 1981). Unlike the UV procedure applied by Sharifi et al. (2007a), the KCl soil extract procedure used in this study replicated the technique applied by Bushong et al. (2007), and removed NO_3–N with the addition of Devarda's alloy for analysis at the 210-nm range. However, the most significant and strongest relationships for both UV_260 and UV_210 was with Cold_KCl$_{NO3}$ ($r = 0.80$ and 0.76, respectively; Table 4). Based on the observations of Norman and Stucki (1981) and Bushong et al. (2007) the unexpectedly high correlation of UV_210 with Cold_KCl$_{NO3}$ found in this study could be due to partial reduction of NO_3–N and NO_2–N by Devarda's alloy. With UV highly correlated to NO_3–N levels, the perception that UV_260 is only related to labile organic forms of N (Sharifi et al., 2007a) is unreliable using the KCl extraction method in this study.

Similar to the findings of Schomberg et al. (2009), TN and TC were highly related in this study ($r = 0.97$, $p < 0.0001$; Table 4). Soil organic matter (SOM) was more highly correlated with TN and ISNT-N ($r > 0.86$) than with TC ($r = 0.78$) (Table 4). Overall inorganic N indices had no significant correlation with SOM (Table 4). The ratio of TC/TN (C/N) is important as it controls N mineralization or immobilization processes in the soil (Whitehead, 1995) and hence soil N supply. The most significant ($p < 0.0001$) N indices that related to C/N were TC and SOM ($r = 0.66$ and 0.61, respectively) followed by ISNT-N ($r = 0.47$, $p < 0.01$), TN ($r = 0.46$, $p < 0.01$) and Fl_CO$_2$ (r

= 0.39, $p < 0.05$). It would be expected that C/N would be positively correlated with TC, TN, and SOM in the soil, but the moderate correlations with ISNT-N and Fl_CO$_2$ indicates that the C/N may also be regulating the labile soil N pools and N supply through mineralization.

Soil texture can influence the proportion of SOM mineralized (particularly under controlled conditions; Griffin, 2008), therefore correlation with a chemical N index may indicate the sensitivity of the test to changes in soil structure and its influence on the N mineralization potential of the soil. Moderate to strong correlations were observed between SOM, TN, and TC with soil textual properties, (g kg^{-1} of sand, silt and clay). Soil organic matter had strong correlations with sand silt and clay ($r = -0.57$, 0.44, $p < 0.01$ and 0.63, respectively, $p < 0.0001$). Sharifi et al. (2007a) similarly found that soil organic C and N had significant correlations with clay content in the soil ($r = 0.72$ and 0.71, respectively, $p < 0.01$). These results are also supported by Hassink (1994b) who found a significant positive relationship between the soil clay plus silt content and soil organic N content of grassland soils ($r^2 = 0.65$). Although lower SOM levels are associated with sandy soils, increased aeration in these soils may lead to higher rates of N mineralization under field conditions (Griffin, 2008). In the current study, soil sand content was negatively correlated with Fl_CO$_2$ and ISNT-N ($r = -0.44$, $p < 0.01$ and -0.43, $p < 0.05$, respectively), while silt content had a similar positive correlation with Fl_CO$_2$ ($r = 0.43$, $p < 0.01$). Whereas sand and silt were more highly correlated with Fl_CO$_2$ than with ISNT-N, clay content was more highly correlated with ISNT-N ($r = 0.48$, $p < 0.01$) than with Fl_CO$_2$ ($r = 0.37$, $p < 0.05$). Similarly, Sharifi et al. (2007a) found that the soil clay content and ISNT-N were strongly correlated ($r = 0.72$, $p < 0.01$). Clay surfaces absorb and bind protein metabolites within SOM and protect them

from rapid microbial degradation through the formation of organic-inorganic complexes (Pinck et al., 1954). The invasive nature of the ISNT works by hydrolysing these structures with a strong alkali to identify the labile N fractions that will be mineralized by microbes under favourable conditions (Khan et al., 2001). A similar process could be considered when using the Hot_KCl_{NH4} and Hyd_N soil extraction procedures, as weak correlations ($p < 0.05$) were also found between these N indices and the soil clay content ($r = 0.36$ and 0.35, respectively). Sharifi et al. (2007a) also reported similar weak to moderate correlation between Hyd_N and clay ($r = 0.50$). In the present study no other chemical N indices displayed significant correlations with soil textual properties.

Relationships between Mineralizable Nitrogen, Soil Nitrogen Indices, and Properties

The ISNT-N displayed the strongest correlation with MN ($r = 0.83$, $p < 0.0001$; Table 4), and in a least squared linear regression explained 69% ($p < 0.0001$) of the variation in MN (Table 5), greater than any other N index. Total N, TC, and SOM explained between 61 and 67% variation in MN across the 35 soil sample data set (Table 5).

Van Eekeren et al. (2010) found similar or slightly lower correlations between MN (measured by AI-7) and TN, TC and SOM ($r = 0.83$, 0.61 and 0.62, $p < 0.05$, respectively) in 20 Dutch permanent grassland soils. Schomberg et al. (2009) also found similar although slightly higher correlations between MN (measured by AI-7) and TN and TC ($r = 0.86$ and 0.84, $p < 0.0001$, respectively). In this study, the C/N ratio was poorly correlated to MN ($r = 0.43$, $p < 0.05$). Sharifi et al. (2007a) also found strong relationships between N_o using a 24-wk aerobic incubation and total organic N and C ($r^2 = 0.67$ and 0.60, $p < 0.001$, respectively), which were greater than the relationship

with ISNT-N ($r^2 = 0.51$, $p < 0.001$). However, Sharifi et al. (2007a) stated that total organic N and C represent characteristic properties of soil that indicate long-term changes in N_o, and would therefore be less sensitive to short-term changes affected through management practices. In the current study TN, TC, and SOM had stronger correlations with ISNT-N and Fl_CO_2 than with MN ($r > 0.71$, Table 4), therefore suggesting that the size of TN, TC and SOM content does not necessary relate to the quantity of MN in these soils. A more sensitive soil test, like the ISNT, which measures a labile fraction of TN may be a better indicator of MN over shorter time frames (i.e., between seasons, or yearly).

The values of MN were moderately related to UV_210, UV_260 and Fl_CO_2, as they explained between 31 and 47% of variability in MN (Table 5). The reliability of the UV measurements (KCl extraction method) for estimating MN is questionable as they were highly correlated with $Cold\_KCl_{NO3}$. However, the correlation between MN and FL_CO_2 suggests that this test could be considered as a possible rapid indicator of the MN capacity of temperate soils. The FL_CO_2 method is different from other N indices evaluated as it does not involve the use of dangerous chemicals through extraction and/or hydrolysis, but rather reflects microbial responses to organic matter mineralization following rewetting of dried soil (Kieft et al., 1987). Relationships between MN and Hot_KCl_{NH4} or Hyd_N were much weaker ($r^2 = 0.24$ and 0.22, respectively; Table 5), indicating that these N indices are not sensitive enough to measure the labile organic components related to the MN capacity of these temperate grassland soils. There was no significant correlation between MN and $KMnO_4\_oxd\_N$ and between MN and indices of inorganic N ($Cold\_KCl_{NH4}$ and $Cold\_KCl_{N03}$; Table 4 and Table 5). The anoxic conditions created using AI-7 would have inhibited

Table 5. Linear regression equations† of mineralizable N (MN) versus soil N indices across 35 temperate mineral soils.

N index‡	Intercept		Slope		P > F§	RMSE¶	r^2
	Estimate	Standard error	Estimate	Standard error			
TN	22.63	28.06	48.39	5.90	<0.0001	45.18	0.67
TC	66.79	24.09	3.66	0.47	<0.0001	46.73	0.65
SOM	29.72	30.99	1.94	0.27	<0.0001	49.2	0.61
$Cold\_KCL_{NH4}$	248.01	20.03	−1.04	4.05	0.7982	78.68	0.00
$Cold\_KCL_{NO3}$	215.50	21.48	1.40	0.84	0.1064	75.67	0.08
Hot_KCL_{NH4}	142.20	33.93	2.00	0.62	0.003	68.81	0.24
Hyd_N	155.78	30.88	1.86	0.60	0.004	69.35	0.22
$Acid\_Oxd\_N$	182.59	84.45	0.45	0.61	0.4658	78.12	0.02
UV_260	83.72	36.82	369.34	81.28	<0.0001	61.77	0.39
UV_210	161.46	24.17	469.58	122.02	0.0005	65.44	0.31
Fl_CO2	−169.60	77.03	6.94	1.28	<0.0001	57.32	0.47
ISNT-N	3.38	29.32	0.68	0.08	<0.0001	44.14	0.69

†Equations are of the form $Y = intercept + slope(x)$, where x is the measured N index, and the intercept and slope were estimated by regression.

‡TN, total soil nitrogen; TC; total soil carbon; SOM; soil organic matter; $Cold\_KCl_{NH4}$, KCl extractable NH_4–N; $Cold\_KCl_{NO3}$, KCl extractable NO_3+NO_2–N; Hot_KCl_{NH4}, heated KCl extractable NH_4–N; Hyd_N, Hydrolyzable N determined as the difference between hot and cold extractable NH_4–N; $KMnO_4\_oxd\_N$, potassium permanganate oxidation measured as NH_4–N; UV_260, ultraviolet absorption of KCl extract at 260 nm wavelength; UV_210, ultraviolet absorption of KCl extract at 210 nm wavelength; Fl_CO_2, flush of carbon dioxide in 24 h; ISNT-N, Illinois Soil Nitrogen Test N.

§ Observed levels of significance probability ($P > F$) of the slopes and intercepts.

¶ RMSE, Root mean square error.

nitrification of NH_4 to NO_3 (Bronson, 2008), and explains why there was no significant relationship between MN and the index of soil NO_3–N (Cold_KCl$_{NO3}$).

Soil textural properties (sand, silt, and clay) have been found to be associated with mineralizable N (Nyiraneza et al., 2012). Sharifi et al. (2007a) found soil clay contents explained 47% of potentially mineralizable N after using a longer 24-wk aerobic incubation period and similarly Nyiraneza et al. (2012) found strong correlations between N_o and soil clay ($r = 0.77$) but slightly poorer correlations with silt ($r = 0.43$). In this study, sand displayed a moderately negative correlation with MN ($r = -0.50, p < 0.01$). The trend was opposite for silt and clay content in soil, where MN levels increased as silt ($r = 0.43$) and clay ($r = 0.50$) content increased. However, compared to the relationship between MN and sand ($r^2 = 0.25, p < 0.01$), the relationship between MN and silt and MN and clay were somewhat weaker ($r^2 = 0.18$ and 0.25, $p < 0.05$, respectively). This could be explained by limited activity of N mineralizing biology over shorter time periods (7 d), and increased protection of organic N within the SOM-clay and silt complexes, which may further reduce microbial decomposition levels within fine textured soils (Pinck et al., 1954; Hassink et al., 1993).

A number of studies have reported an improvement in predicting mineralized N (as N_o and MN) in soils by combining various N indices and soil properties in multiple regression models (Gallagher and Bartholomew, 1964; Schomberg et al., 2009, Ros et al., 2011b). A mixed multiple stepwise regression analysis was employed in this study to predict MN (dependent variable) where all N indices and soil biogeochemical properties were available for selection as independent variables. However, no combination of methods evaluated and soil properties were an improvement on the linear regression model of ISNT-N (Table 5).

Effect of Textural Homogeneity on Prediction of Mineralizable Nitrogen

The four groups of soil textures identified in this study were classified further into two groups, loamy sand and sandy loam as coarse textured soils ($n = 7$) and loam and clay loam as fine textured soils ($n = 28$). However, when compared against the prediction model using the whole data set ($n = 35$), there was no improvement in the prediction models using each subset of soils based on soil texture. For example the relationship between ISNT-N and MN was poorer for the fine textured soils ($n = 28$, $r^2 = 0.61, p < 0.0001$) (Fig. 1), while, TN by itself was the best predictor of MN ($r^2 = 0.67, p < 0.0001$) for these soils (Fig. 2).

Strong correlations between biological indices measuring potential N mineralization and total N have been found in other studies that just focused on soils of similar texture or geographical region. For example, Nyiraneza et al. (2012), found that TN was positively related to N_o using a 24-wk aerobic incubation in fine textured soils ($r = 0.69, p < 0.01$). Gianello and Bremner (1986) also reported strong corrections between TN and MN ($r = 0.79$) using soils from a similar geographical area. However,
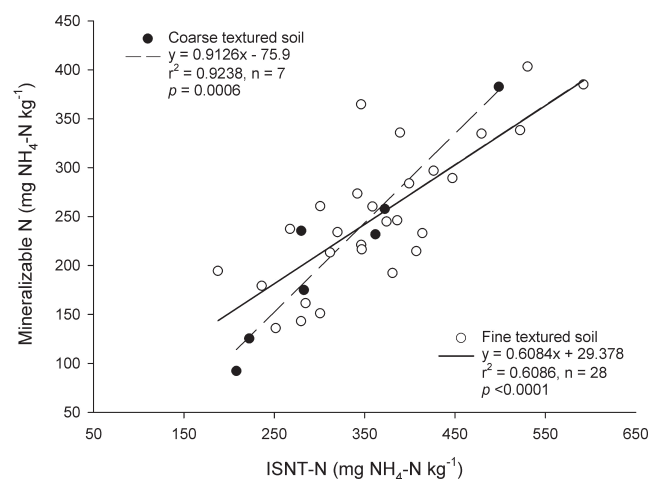

Fig. 1. The relationship between mineralizable N measured using a 7-d anaerobic incubation (AI-7) versus the Illinois soil N test [ISNT-N]) across coarse and fine textured soils.

the ability of TN to predict MN across soil types representing a wide geographical area and reflecting changes in N availability through management practices is questionable, as relatively stable and protected organic N pools make up a large proportion of TN (Hassink, 1994a; Hassink, 1997). Therefore TN would not be expected to be a reliable index of MN across a range of temperate grassland soils which would be under the influence of different environmental and management conditions.

Serna and Pomares (1992) found that chemical N indices were more effective in predicting mineralizable N in sandy soils, suggesting that the microaggregate formations in clay soils made it difficult to extract N fractions, resulting in poorer relationships with MN after a 16-wk aerobic incubation. In the current study there was a much better relationship with MN and ISNT-N in course textured soils ($r^2 = 0.92, p < 0.01$; Fig. 1) compared with fine textured soils and ISNT-N by itself was the best predictor of MN. However, with so few data points across these coarse soils ($n = 7$), which had a wide range in MN ($92–382$ mg kg^{-1}) as measured using AI-7, the prediction of MN using the ISNT would need further investigation based on soil textural class.
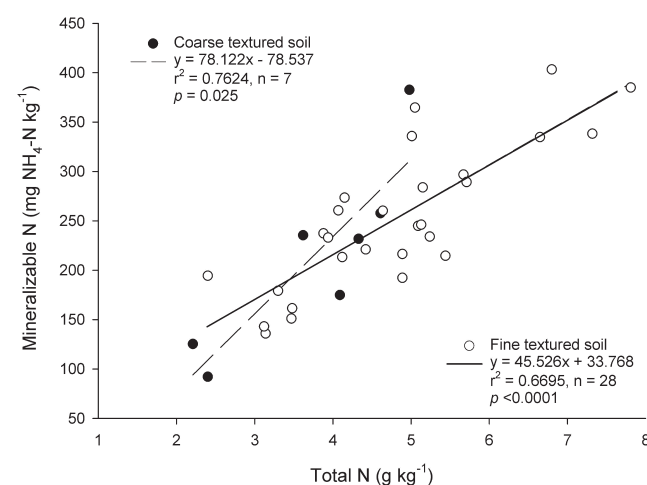

Fig. 2. The relationship between mineralizable N measured using a 7-d anaerobic incubation (AI-7) versus total N across coarse and fine textured soils.

The effectiveness of the ISNT to predict MN across all textural groups demonstrates the robustness of this soil N test and its universal application across temperate soil types. Although ISNT measures a relatively stable form of soil N, it has been shown to detect increases in MN pools related to recent applications of organic manure (Klapwyk et al., 2006). This suggests that the fluctuations in MN during a growing season should reflect the changes in the labile pool of amino sugars.

CONCLUSIONS

A large range in MN as measured by AI-7 exists among different temperate grassland mineral soil types typical to Ireland. This indicates that potential benefits could be accrued by adjusting current N fertilizer recommendations for grassland to account for the N supplied through N mineralization.

This study showed that more rapid chemical N indices were able to predict MN across a range of grassland soils. The ISNT, TN, TC, SOM, and Fl_CO_2 were the most reliable indices explaining > 47% of the variability in MN. The stable nature of TN, TC, and SOM makes these indices less sensitive to soil management practices, such as organic and inorganic fertilizer inputs, and short term soil structural changes caused by soil drying and rewetting typically experienced by temperate climates. Therefore, these indices may not reflect the potentially labile N supply available for plant uptake over a growing season, but do indicate changes over a longer periods of time (i.e., years). Following a stepwise regression analysis of all the N indices evaluated in this study, the ISNT by itself was the most effective soil N test for predicting MN across a wide range of productive temperate grassland mineral soil types. Grouping of these soils based on similar textural properties did not demonstrate any significant improvement by these N indices in predicting MN. However, further evaluation over a larger dataset of temperate soils with similar textural properties could warrant further investigation in future studies.

Given the simplicity, reliability, and robustness of the ISNT it shows potential as a routine test for predicting MN. Further development of the ISNT for this purpose could enable more precise N fertilizer recommendations to be developed for grassland soils and could improve N_fUE on farms and reduce N losses and the associated environmental impacts. Additional research is required to validate the effectiveness of ISNT in predicting N supply for temperate grassland across varying climatic conditions, temporal seasonal changes, and soil management practices.

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