Rapid Assays to Predict Nitrogen Mineralization Capacity of Agricultural Soils

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Inability to predict the quantity of nitrogen a soil can supply via mineralization remains a serious obstacle to the improvement of N management. A large-scale study (130 soils) was conducted to identify laboratory assays that may enable N mineralization potential of New Zealand soils to be estimated reliably and rapidly. To ensure that the study delivered robust conclusions, samples (0–15 cm) were collected from a wide range of sedimentary and allophanic soils representing different land uses (pastures; arable cropping). The selected assays included: N mineralized in a 7-d anaerobic incubation at either 40 or 25°C; CO_2–C evolved in 24-h following re-wetting of air-dry soil ("CO_2 burst test"); and N mineralized in a 2-wk aerobic incubation. Dissolved organic matter was determined using "mild" extractants: cold and hot water and 0.01 mol L^{-1} $NaHCO_3$. Particulate organic matter was included as it is known to be labile and can be rapidly quantified. These assays were evaluated against N mineralization potential measured in a 14-wk aerobic incubation at 25°C. The assays that correlated closely with mineralization potential included anaerobically mineralizable N and CO_2 burst test values. Particularly strong correlations were obtained for hot water extractable N, suggesting that this easily-measured organic N fraction can be used to predict N supply potential across a wide range of soil types and land uses. Inclusion of hot water specific ultraviolet absorbance (260 nm) in a multivariate regression with hot water extractable N and land use produced the best-fit model for explaining variability in N mineralization potential.

Abbreviations: AMN, anaerobically mineralizable N; ASC, anion storage capacity; HWEON, hot water extractable organic N; POM, particulate organic matter; SUVA, specific ultra violet absorbance.

Core Ideas

- **Mineralized soil N is an important source of N for crops, but difficult to predict.**
- **A reliable, "laboratory-friendly" test for mineralizable N is not yet available.**
- **Chemical and bioassays were assessed to identify a "quick-test" for mineralizable N.**
- **Assays were evaluated against N mineralized in 14-week incubation (130 soils).**
- **Best tests: hot water-extractable N or CO_2 evolved after re-wetting air-dry soil.**

Nitrogen (N) is the most common growth-limiting nutrient in agricultural production. To meet the shortfall between soil N supply and crop requirements, fertilizer N use increased worldwide from 4 to 83 Tg yr^{-1} between 1950 and 2000 (Erisman et al., 2008). At the same time, N losses to water and air have increased substantially, leading to environmental, ecological, and human health problems (Galloway et al., 2008). Ongoing public concern over environmental damage, including contamination of groundwater with nitrate N originating in agricultural land, has increased the pressure on farmers and regulatory authorities to improve N management practices. The most direct approach to improve N use efficiency is to match N inputs to crop N requirements. Fundamental to the success of this approach is an ability to estimate the N supplied during the growing season through mineralization of soil organic matter.

The contribution of mineralized N to crop N supply can vary widely, depending on the quantity of mineralizable organic matter in the soil and the environmental conditions (especially soil temperature and moisture) that regulate the rate of mineralization (Curtin et al., 2012; Dessureault-Rompré et al., 2015; Goh, 1983; Sakadevan et al., 1993). Estimating soil N mineralization rates during the grow-

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ing season has proved difficult, in large part because the pool of mineralizable soil N (i.e., potentially mineralizable N) is not readily amenable to quantification on a routine or timely basis. The most widely accepted measure of potentially mineralizable N involves a laboratory bioassay in which mineral N release is quantified from soil maintained under optimum (and constant) environmental conditions over an extended time period (20+ weeks) (Stanford and Smith, 1972).

There has been a substantial research effort to identify assays or tests that would enable N mineralization potential to be estimated rapidly and with an acceptable level of confidence (Curtin and McCallum, 2004; Curtin and Wen, 1999; Ros et al., 2011a, 2011b; Sharifi et al., 2007). These existing assays can be grouped into a number of broad categories: (i) bioassays in which short-term mineralization of N (or C) is measured; (ii) extraction procedures for organic N (and/or C) using chemical reagents that differ in extraction "intensity"; and (iii) methods that quantify soil organic matter fractions that are known to be labile (e.g., particulate organic matter, light fraction organic matter).

Possibly the most widely used bioassay is that of Keeney and Bremner (1966), which provides a measure of N mineralization under anaerobic conditions during a week-long incubation at 40°C. While this assay is operationally suited to routine soil testing, measurement conditions (anaerobicity and high temperature) make agronomic interpretation of this test uncertain unless field calibration has been performed (Christensen and Mellbye, 2006). Despite this uncertainty, it is sometimes used as the method against which other indices of N mineralization are referenced (McDonald et al., 2014).

Nitrogen mineralized in a short-term aerobic incubation (e.g., 2 wk at 25°C) has shown promise as a predictor of N mineralization potential and of N supply under field conditions (Campbell et al., 1994). When air-dry soil is used in this assay, part of the measured N results from the flush of mineralization that occurs on re-wetting (Birch effect). Although this flush of mineralization is often attributed to the soil microbial biomass (Sparling and Ross, 1988), Appel (1998) presented evidence that the organic N released on re-wetting originates from non-biomass organic matter. The respiration flush from re-wetted soils ["CO_2 burst"; (Franzluebbers et al., 2000; Haney and Haney, 2010; Haney et al., 2001)] has been proposed as an alternative predictor of N mineralization potential (Schomberg et al., 2009).

Numerous chemical extraction procedures have been evaluated as predictors of mineralization potential. Chemical methods typically involve measuring organic N and/or ammonium N in an extractant such as water or salt solution. The amount of N extracted depends on factors such as salt type, molarity, temperature and duration of extraction, pH, and soil to solution ratio (Chantigny et al., 2010, 2014; Curtin et al., 2015; Ros et al., 2011a, 2011b). From a meta-analysis of published data, Ros et al. (2011b) showed that many extraction methods were no better than total soil N as predictors of mineralizable N. Among the extractants that were superior to total N were cold and hot water. However, Ros et al. (2011b) expressed doubt that any one extraction procedure would provide accurate predictions of mineralizable N across a broad range of soil types and management histories. They recommended a "multi component" approach in which mineralizable N is estimated using a combination of soil tests and site-specific information such as land use and soil texture.

The N mineralization test offered by commercial laboratories in New Zealand is anaerobically mineralizable N, measured in a 7-d incubation at 40°C based on the method described by Keeney and Bremner (1966). However, this test is regarded as having limited diagnostic and/or predictive value (it may allow soils to be ranked in broad categories of "high", "medium", "low" mineralization potential) (Curtin and McCallum, 2004). Recently, total soil N (in the top 7.5 cm) has been proposed as an alternative test to predict fertilizer N responsiveness of New Zealand pastures (Shepherd et al., 2015). Our objective was to identify an assay (or a combination of assays) that would provide reliable predictions of N mineralization potential in New Zealand soils that are diverse in terms of physicochemical characteristics and management history.

MATERIALS AND METHODS

Soil Sampling and Characterization

Soil samples were collected from 130 sites in major agricultural regions of the North (Waikato, Auckland, Hawkes Bay and Gisborne) and South (Canterbury and Southland) Islands of New Zealand. The sites sampled represented soils derived from both sedimentary and volcanic parent materials and soils with different management histories (soils under dairy and drystock pastures and soils used for arable and vegetable cropping). The soils also represented important orders in the New Zealand Soil Classification System: i.e., Brown (Inceptisols, Alfisols in USDA Soil Taxonomy); Pallic (Inceptisols, Alfisols); Recent (Entisols, Inceptisols); Gley (Aquic groups); and Allophanic (Andisols) soil orders. At each sampling site, four soil cores (7-cm diam.) were collected to a depth of 15 cm and composited (total ~3 kg soil collected). In the laboratory, the soils were sieved (4 mm) and air dried at 25°C.

Physico-chemical characteristics of the soils were determined as follows: pH was measured at a soil to water ratio of 1:2 using a standard glass electrode; total C and N were determined by Dumas combustion (LECO TruMac, Leco Corporation, St. Joseph, MI); and particle size distribution was determined by sieving and sedimentation (Gee and Or, 2002) following soil dispersion by sonication. Anion storage capacity (ASC) was measured by the procedure of Saunders (1965), which is designed to saturate the soil with phosphate. This is achieved by shaking soil with a NaOAc-acetic acid solution (buffered at pH 4.6) containing a high concentration of P (5000 mg P kg^{-1}) for 24 h. The ASC value (i.e., P retained as a percentage of added P) can be considered a surrogate for free Al and Fe (Saunders, 1965).

Mineralization Potential

The potential of the soils to mineralize N was assessed in a 14-wk aerobic laboratory incubation. Samples of air-dry soil

(equivalent to 25 g of oven-dry soil) were weighed into plastic vials (50 mL) and deionized water was added drop-wise (using an electronic pipette in titrate mode) to adjust soil water content to 90% of field capacity (field capacity defined as water content at -10 kPa; measured using a tension table). The soils were incubated at 25°C. To minimize moisture loss during incubation, the vials were covered with film (holes were punctured in the film to facilitate aeration). Water was added, if required, at weekly intervals to compensate for any evaporative losses. Mineral N was extracted (by 2 mol L^{-1} KCl) after 2, 4, 7, 10, and 14 wk of incubation and determined using an automated colorimeter (QuickChem 8000 FIA+, Lachat Instruments, Loveland, CO). We incubated a sufficient number of subsamples of each soil to allow one subsample to be destructively sampled at each time point. Mineralized N was estimated by subtracting mineral N at the start of the incubation from the amount determined at each incubation interval.

Carbon mineralization was measured in soil (25 g oven-dry soil equivalent) incubated (in 1-L air-tight jars, fitted with rubber septa) at 25°C after soil wetting as described above. Using a gas-tight Hamilton syringe, fitted with a non-coring needle, the headspace air was periodically sampled (total of 17 samplings during the 14 wk incubation; i.e, on Days 1, 3, 7, 10, 14, 21, 28, 35, 42, 49, 56, 63, 70, 77, 84, 91, and 98). At each sampling, the headspace was mixed by pumping the syringe three times before withdrawing a 20-mL sample of headspace air. The CO_2 concentration was determined using an infrared gas analyzer (LI-COR, Lincoln, NE). After analysis the jars were opened and flushed with fresh air to return CO_2 concentrations to ambient levels before replacing them in the incubator. During the incubation, the soils were weighed periodically and deionized water was added as required to compensate for evaporative losses, as for the N mineralization assays.

Biological Assays

Anaerobically mineralizable N (AMN) was determined as the amount of N generated during a 7-d anaerobic incubation (Keeney and Bremner, 1966). A 5-g soil sample was weighed into a 50-mL centrifuge tube and, after adding 10 mL of deionized water, the soil-water suspension was incubated at 40°C (the tubes sealed during incubation; headspace volume was 35 mL). After incubation, ammonium was extracted in 2 mol L^{-1} KCl and determined using an automated colorimeter as described above. The AMN value was calculated by subtracting ammonium N in the soil prior to incubation from the amount measured after incubation. A second measurement of AMN was performed at a temperature of 25°C; otherwise the incubation procedure was identical to that described for the 40°C assay.

The "CO_2 burst test" involved measuring the amount of CO_2–C evolved after re-wetting air-dry soil (Franzluebbers et al., 2000).The soils were wetted using a capillary procedure similar to that described by Haney and Haney (2010). A 40-g sample of air-dry soil was weighed into a 60-mL vial that had holes (5.5-mm diam.) drilled into the base to allow water entry (the base was covered with a glass microfilter before adding the soil). Each vial (with soil) was placed in a 1-L glass jar containing 20 mL of water; uptake of water by the soils was rapid and visual inspection indicated that water reached the sample surface within ~15 min. The jars were sealed and incubated at 25°C for 24 h, after which a headspace sample was taken for CO_2 determination. The jar lids were fitted with rubber septa to facilitate headspace sampling. The CO_2 concentration was determined using an infrared gas analyzer.

Particulate Organic Matter

Particulate organic matter (POM, i.e., organic matter in the >50-μm particle size fraction; (Cambardella and Elliott, 1992; Gregorich et al., 2006) was separated after dispersion of soil (20 g) by sonication (Qiu et al., 2010). The dispersed soil was passed over a set of sieves (250 and 50 μm) and material on the sieves was washed using a stream of water until the draining water was clear. The materials retained on the 250- and 50-μm sieves (i.e., coarse and fine particulate organic matter, respectively) were dried at 60°C and weighed. The >250-μm and the 250- to 50-μm fractions were individually homogenized and ground using a mortar and pestle and analyzed for C and N using a LECO C/N analyzer. Values for total POM-C and POM-N (sum of the two fractions) are presented in this paper.

Extraction Procedures

Extractable organic matter was determined using several "mild" extractants (0.01 mol L^{-1} $NaHCO_3$; water at room temperature, 20 ± 1°C; and hot water). In the bicarbonate procedure, soil (2.5 g) was shaken with 50 mL of 0.01 mol L^{-1} $NaHCO_3$ on an orbital shaker for 15 min (Fox and Piekielek, 1978; Hong et al., 1990). The suspensions were centrifuged and the supernatants filtered using pre-leached Munktell 393 filter papers. Ultraviolet (UV) absorbance at 260 nm was determined on a Molecular Devices SprectMax M2 plate reader using Greiner Bioone UV-Star 96-well plates and a 1-cm cell. Non-purgable organic C was measured in the extracts using a total organic carbon analyzer (Shimadzu TOC-$V_{CSH,}$ Shimadzu Corp, Japan).

Water extractable N and C were determined as described by Curtin et al. (2006) and Ghani et al. (2003). In a preliminary step, readily soluble organic matter was removed by extracting with deionized water at room temperature (20 ± 1°C; hereafter referred to as *cold water* extraction). This involved shaking 4-g soil samples with 40 mL of deionized water in 50-mL centrifuge tubes for 30 min. The soil-water suspension was then centrifuged, and the supernatant filtered through a pre-leached filter paper (Munktell 393). The centrifuge tube plus wet soil was weighed to calculate the entrained water volume. Another 40-mL aliquot of water was added and, after mixing to resuspend the soil, the tubes were placed in a water bath at 80°C for 16 h. The tubes were then centrifuged and the supernatant solution collected after filtration. Dissolved organic C in the cold and hot water extracts was determined using a total organic carbon analyzer (Shimadzu TOC-$V_{CSH,}$ Shimadzu Corp, Japan). Total N

was determined by persulfate oxidation, as described by Cabrera and Beare (1993), and dissolved organic N was estimated by subtracting mineral N (NH_4^+ and NO_3^- determined using an automated colorimeter) from total N. The UV absorbance of the water extracts was determined at 260 nm, as for the bicarbonate extracts. Specific UV absorbance (SUVA) was calculated by dividing absorbance (cm^{-1}) by the concentration of dissolved organic C ($mg\ L^{-1}$).

Hot water-extractable N and C were also determined without the cold water pre-treatment. After the 16-h extraction with water at 80°C (4 g soil; 40 mL water), the soil-water suspension was centrifuged and the supernatant collected (after filtration) and analyzed, as described above. The soil was then extracted with 2 mol L^{-1} KCl. This step was included to ensure that all of the ammonium N generated during the 16-h hot water extraction was recovered (previous work showed that a large part of the ammonium N generated during hot water extraction adsorbs onto soil cation exchange sites (Curtin et al., 2006)). Hereafter, *hot water* extractable ammonium N will refer to the combined amount of ammonium N in the hot water and the subsequent 2 mol L^{-1} KCl extract (less ammonium N in the soil prior to hot water extraction).

Statistical Analyses

Data were analyzed using a mixed model fitted with restricted maximum likelihood (REML) to account for the lack of balance in the data. An indication of the variation associated with estimated means is provided by the least significant difference (LSD) at the 5% level, and an asymptotically large denominator degrees of freedom. Relationships between measured variables were examined using linear regression and correlation analysis.

RESULTS

Properties of Soils: Influence of Land Use and Soil Type

Of the 130 sites sampled, 113 were on sedimentary soils and 17 were on allophanic soils (Table 1). Forty-six sites (all on sedimentary soils) had a history of arable cropping and 55 sites were under pasture (31 of the pasture samples were collected on dairy farms and 24 from drystock enterprises). In addition to fields under grazed pastures, a small number of sites (8) were un-grazed grassland (mostly reserves and parks, with minimal inputs of fertilizer N). Of the 21 fields under vegetable cropping, 8 were on allophanic soils (in the Waikato and Auckland regions) and 13 on sedimentary soils.

The soils represented a wide range of organic matter (total C ranged from 13 to 88 g kg^{-1}) and textures (65 to 425 g clay kg^{-1}; 10 to 453 g sand kg^{-1}). The total C and N content was significantly ($P < 0.001$) greater in pastoral soils compared with soils under arable or vegetable cropping and greater in allophanic than in sedimentary soils (Table 1). Particulate organic matter comprised 12.5 ± 4.1% (mean ± standard deviation) of total soil C but a smaller proportion (9.8 ± 3.9%) of soil total N. The C to N ratio of POM (14.1 ± 1.8) was wider than that of soil organic matter as a whole (10.7 ± 1.0). The proportion of POM C and N was greater ($P < 0.001$) in pastoral soils than in arable and vegetable soils (Table 1). Allophanic soils had a significantly ($P < 0.001$) larger proportion of POM-C and POM-N than sedimentary soils (on average, POM-C comprised 18.2% of soil C in allophanic soils under pasture vs. 14.5% in sedimentary soils under this land use).

As expected, allophanic soils had much higher ASC values than sedimentary soils (mean of 96% for allophanic soils vs. 32% for sedimentary soils). The differences in pH among soil types and land uses were generally small (mean pH ~6 for all soil type × land use combinations). The allophanic soils had relatively low clay content (and high sand content); differences in clay content between land uses were not significant.

Nitrogen Mineralization Potential

Total N mineralized in the 14-wk incubation (i.e., N mineralization potential) ranged from 45 to 393 mg kg^{-1} (mean 144 mg kg^{-1}). Mineralization of N and C was rapid in the first 2 wk of incubation after which it proceeded at a more gradual rate (Fig. 1). About half (52%, on average) of total mineraliza-

Table 1. Soil physicochemical properties (pH, texture, anion storage capacity [ASC]) and total and particulate organic C and N in sedimentary and allophanic soils under different land uses.

Land use	Paddocks sampled	pH	Sand	Clay	ASC	Total C	Total N	POM-C†	POM-C/TC‡	POM-N	POM-N/TN§
			%			g kg^{-1}			%	mg kg^{-1}	%
						Sedimentary					
Dairy	24	6.0	23	22	46	49	4.4	7.5	15	571	12
Drystock	22	6.0	20	26	34	41	3.7	6.0	14	442	12
Arable	46	6.0	17	26	24	26	2.5	2.5	9	170	7
Vegetable	13	6.3	19	27	35	25	2.4	2.5	9	172	7
Ungrazed	8	6.2	18	26	22	38	3.5	6.6	17	439	12
						Allophanic					
Dairy	7	6.0	32	12	96	75	7.0	13.0	17	1046	15
Drystock	2	5.9	29	11	95	77	7.0	15.7	21	1210	18
Vegetable	8	6.2	35	11	95	50	4.8	6.1	12	506	11
LSD (5%)		0.26	9.5	6.3	14.5	10.3	0.8	2.3	2.7	170	2.6

† POM, particulate organic matter.

‡ TC, total carbon.

§ TN, total nitrogen.

tion occurred during the first 2 wk of incubation. Comparison of the amounts of N and C mineralized indicated that the organic matter mineralized in the early phase of the incubation was N-rich (Table 2). The mean ratio of CO_2–C evolved to N mineralized in the first 2 wk was 6.7 ± 1.5 compared with 9.2 ± 1.7 for the entire 14-wk incubation. The ratio was lower in soils under pastures than in soils used for arable and vegetable cropping (Table 2).

Land use history had a dominant influence on N mineralization potential, with pastoral soils mineralizing much more N than soils used for arable or vegetable cropping (Fig. 1). Nitrogen mineralized in 14 wk was 203 ± 62 mg kg^{-1} in pastoral soils vs. 89 ± 25 mg kg^{-1} in cropped soils. Considerable variation in mineralization potential existed within both the pastoral and cropping soil groups (Fig. 1). However, differences between dairy and drystock pastures were not significant overall, nor was there a significant difference between soils used for arable and vegetable cropping (Table 2).

Although the allophanic soils, which had a large proportion of POM-N (Table 1), might be expected to mineralize a larger proportion of soil N than sedimentary soils, the reverse was the case; a significantly (P < 0.001) smaller fraction of total soil N mineralized in allophanic vs. sedimentary soils (2.7 ± 0.5% vs. 4.4 ± 1.3%).

Bioassays

As with N mineralized in the 14-wk aerobic incubation, a wide range of values (8 to 213 mg N kg^{-1}; mean 84 mg kg^{-1}) were obtained when anaerobically mineralizable N (AMN) was measured at 40°C. AMN was strongly influenced by land use (pasture > arable or vegetable cropping) and allophanic soils tended (P = 0.10) to have higher values than sedimentary soils (Table 2). The AMN values measured at 40 and 25°C were strongly correlated (r = 0.90; P < 0.001) and, as expected, AMN decreased when measurement temperature was reduced from 40 to 25°C (mean of 46.1 at 25°C vs. 83.9 mg kg^{-1} at 40°C). The AMN value at 25°C was greater for pastures than for cropped soils (P < 0.001), but soil type did not have a significant effect (P = 0.20).

The rate of CO_2–C evolution in the 24-h period after re-wetting dry soil (CO_2 burst test) ranged from 1.6 to 12.5 mg kg^{-1} per hour (mean 5.0 mg kg^{-1} h^{-1}). Values for pasture soils were generally 2 to 3 times those for cropped soils (Table 2). Sedimentary soils used for dairying had higher burst test values than allophanic soils under dairy pastures; in the other land use categories, there was no significant difference between allophanic and sedimentary soils.

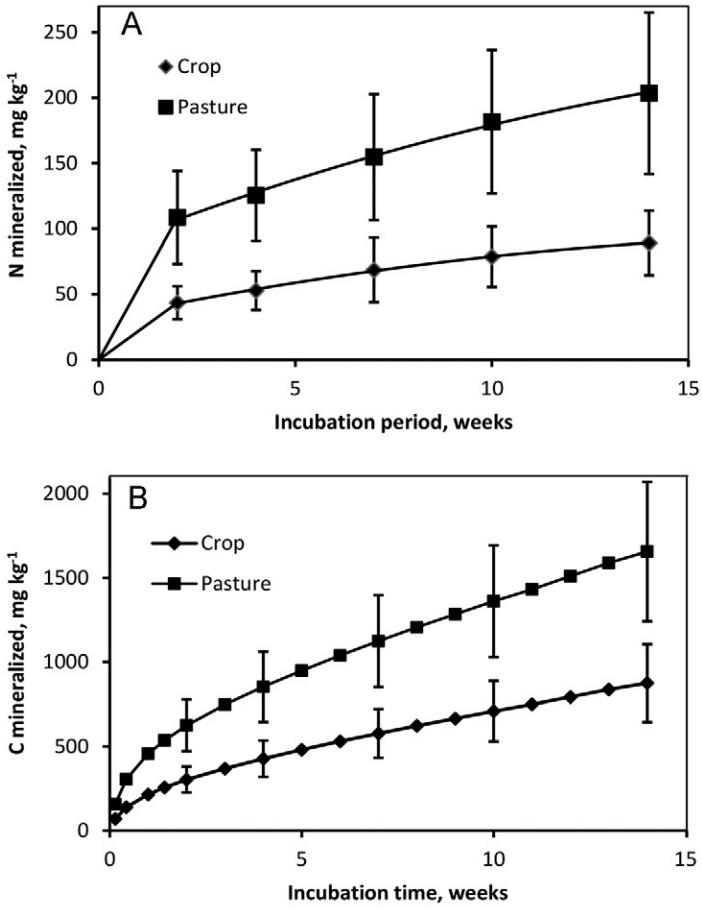

Fig. 1. Cumulative amounts of mineralized N (A) and mineralized C (B) in cropping (arable and vegetable) and pastoral (dairy and drystock) soils during a 14-wk incubation at 25°C. Error bars represent 2 standard deviations.

Extractable Organic Matter

Cold water and 0.01 mol L^{-1} $NaHCO_3$ extracted similar quantities of organic matter (260 ± 101 mg C kg^{-1} for cold water vs. 292 ± 115 mg kg^{-1} for $NaHCO_3$) (Table 3). Organic N extracted in cold water was small (between 7 and 51 mg kg^{-1})

Table 2. Effect of land use and soil type on N mineralized in aerobic incubation (in first 14 d and in entire 14 wk), anaerobically mineralizable N (AMN), and on CO_2 release following re-wetting of air-dry soil (CO_2 burst).

Land use	N mineralized		C min/N min ratio†		N mineralized in 14 wk	AMN		CO_2 Burst
	2 wk	14 wk	2 wk	14 wk		40°C	25°C	
	mg kg^{-1}				% of total N	mg kg^{-1}		mg C kg^{-1} h^{-1}
				Sedimentary				
Dairy	114	216	5.8	7.9	5.1	122	82	7.7
Drystock	101	194	6.3	8.4	5.2	112	68	6.8
Arable	45	91	7.1	9.8	3.2	52	23	3.3
Vegetable	38	77	7.1	10.6	3.7	38	13	2.8
Ungrazed	94	194	7.2	9.1	5.7	133	75	7.3
				Allophanic				
Dairy	123	213	5.9	9.3	3.1	137	71	6.2
Drystock	121	213	6.4	9.0	3.0	141	77	6.1
Vegetable	51	105	7.7	10.4	2.2	56	21	3.1
LSD (5%)	23.8	41.2	1.3	1.4	0.96	29.5	19.1	1.31

† Ratio of CO_2–C evolved to N mineralized in the first 2 wk and in entire 14 wk of aerobic incubation at 25°C.

Table 3. Effect of land use and soil type on amounts of C and N extracted using 0.01 mol L^{-1} $NaHCO_3$, and cold (room temperature, 20 ± 1°C) and hot (80°C) water.

Land use	$NaHCO_3$–C	CWEC†	CWEON	HWEC (single ext.)	HWEON (single ext.)	HW NH_4–N	HWEC/TC	HWEON/TN	SUVA
	mg kg^{-1}						%	%	L mg C^{-1} cm^{-1}
				Sedimentary					
Dairy	389	355	23.4	2305 (2227)	203	39 (16)	4.9	4.7	0.012
Drystock	329	316	21.6	1882 (1822)	170	32 (16)	4.7	4.5	0.012
Arable	220	197	14.1	1062 (1071)	89	17 (16)	4.0	3.6	0.016
Vegetable	207	189	14.0	875 (940)	72	15 (18)	3.5	2.9	0.021
Ungrazed	317	333	25.9	1994 (1927)	181	35 (16)	5.4	5.2	0.015
				Allophanic					
Dairy	466	337	21.6	2363 (2244)	197	57 (23)	3.1	2.7	0.011
Drystock	435	331	17.3	2047 (1944)	156	52 (26)	2.6	2.2	0.010
Vegetable	260	162	10.0	1142 (1141)	91	31 (26)	2.3	1.9	0.012
LSD (5%)	78	67	5.4	466	40		0.78	0.79	0.003

† Abbreviations: CWEC, cold water extractable C; CWEON, cold water extractable organic N; HWEC, hot water extractable C (values in brackets are amounts of C removed by sequential extraction with cold water followed by hot water); HWEON, hot water extractable organic N; HW NH_4–N, ammonium N generated by hot water extraction (includes NH_4–N in the hot water extract plus NH_4–N in subsequent 2 mol L^{-1} KCl extract). Values in brackets represent HW NH_4–N as a proportion of all N (NH_4–N plus organic N) generated by hot water extraction; TC, total C; TN, total N; SUVA, specific UV absorbance measured in the hot water (single) extract at 260 nm.

relative to N mineralized during the 14-wk incubation (45 to 393 mg N kg^{-1}). Subsequent extraction with hot water removed 5.8 times as much organic N, on average, as cold water (results not shown). Amounts of organic matter (soil C) removed by sequential extraction with cold and then hot water were similar to those extracted in hot water alone (Table 3). Between 2.0 and 8.0% (mean 4.1%) of soil C was removed using hot water (single extract) and a slightly smaller proportion of soil N (mean 3.8%; range 1.5 to 7.6%) was recovered as dissolved organic N in the hot water extract.

In addition to organic N, NH_4–N (6 to 76 mg N kg^{-1}) was also released during hot water extraction. Ammonium N represented 16 ± 3% of the N released in sedimentary soils during hot water extraction (organic N plus NH_4–N) but a significantly larger proportion (25 ± 3%) of that generated in allophanic soils. The ammonium N generated during hot water extraction was more closely related with total soil N (r = 0.91) than with hot water extractable organic N (HWEON) (r = 0.84).

Hot water (single extract, no pre-extraction with cold water) removed significantly (P < 0.001) larger proportions of soil C and N from pastoral than from cropped soils and from sedimentary vs. allophanic soils (Table 3). The SUVA of the hot water extracts varied considerably (range 0.008 to 0.030 L mg^{-1} cm^{-1}; mean 0.014 L mg^{-1} cm^{-1}). Values of SUVA were greater for vegetable and arable soils than for soils under grazed pastures (Table 3). There was little difference in the overall mean SUVA values obtained from sedimentary and allophanic soils under dairy and drystock management.

Relationships between Indices of N Mineralization Potential

Indicators of N mineralization potential (extractable N and C; AMN; POM) were significantly (P < 0.001) correlated with total soil N (and C) (Table 4). Particulate organic matter and hot water extractable ammonium N showed particularly strong relationships with total N ($r \geq 0.89$). The two measures of anaerobically mineralizable N showed strong associations with cold and hot water extractable organic N (and C). The AMN tests also correlated strongly with the other bioassay included in the study, the CO_2 burst test (r = 0.87– 0.89). The burst test also showed a strong association with water extractable organic N and C, particularly with HWEON (r = 0.92).

Although the soils included in this study had a wide range of total N content, it accounted for less than half (48%) of the variability in N mineralization potential, measured as the N mineralized in 14 wk of aerobic incubation (Fig. 2). Particulate organic N was less successful than total N as a predictor of N mineralization potential (36% of variability explained by POM-N). Better relationships were obtained with AMN, particularly the 25°C measurement which accounted for 81% of the variability in mineralization potential (compared with 74% for the 40°C measurement). However, for both measures of AMN, but particularly for AMN at 25°C, there were positive (P < 0.001) intercept values (several soils with very low AMN values mineralized significant amounts of N during the 14-wk aerobic incubation).

In previous studies, the concentration of organic matter in $NaHCO_3$ extracts has been assessed by measuring UV absorbance (at 260 nm) (Fox and Piekielek, 1978; Sharifi et al., 2007). In this study, there was a poor relationship between the bicarbonate UV value and N mineralization potential (r = 0.57). The relationship improved when organic C in $NaHCO_3$ was regressed against N mineralization potential (r = 0.85). Cold water extractable organic N explained a similar proportion (62%) of the variability in N mineralization potential as bicarbonate extractable organic C.

The CO_2 burst test was a much better predictor; it accounted for 84% of the variability in N mineralization potential, similar to the proportion of variability explained by N mineralized during the first 2 wk of the aerobic incubation (Fig. 3). The best single indicator was HWEON (88% of variability explained vs. 85% for hot water extractable C). The quantity of HWEON was generally similar to the amount of N mineralized during the 14-wk aerobic incubation. The SUVA values obtained from the hot water extracts were negatively correlated with N mineralization potential (r = -0.47; P < 0.001).

Although hot water extractable ammonium N also showed an association with N mineralization potential, it accounted for much less of the variability (65%) than HWEON. Including hot water extractable NH_4–N in a regression model with HWEON did not significantly improve the relationship with N mineralization potential N (not shown). Combining one other mineralization indicator (either total N, AMN, or the CO_2–C burst test) with HWEON also did not significantly improve the relationship with mineralization potential.

A multivariate regression model that included SUVA and land use terms [soils grouped into pastoral (including 8 ungrazed soils) and cropped categories] along with HWEON provided the best prediction of N mineralization (91% of the variability in N mineralization accounted for; Fig. 4). This model was in the form:

$$y = a + b_1 \text{ HWEON} + \text{LU} + b_2 \text{ SUVA}$$

where y is N mineralization potential, a is the intercept, and b_1 and b_2 are regression coefficients (the unit for N mineralization and HWEON is mg kg^{-1} soil). Parameter estimates (standard errors in brackets) were: a = 16.8 (11.4) and b_1 = 0.86 (0.05). The land use (LU) and SUVA terms, which apply only to the pastoral soils, account for a (small) difference in the relationship between HWEON and mineralization potential in cropped vs. pastoral soils: LU = 82.9 (17.5) and b_2 = –4414 (1300).

Adding terms that reflect soil texture and mineralogy (clay content and ASC) to the model did not significantly improve prediction of mineralization potential.

DISCUSSION

Results obtained from laboratory N mineralization incubations can be influenced by sample handling prior to incubation. As observed in other studies where air-dry soils were used (Beauchamp et al., 1986; Wu and Brookes, 2005), mineralization was particularly rapid in the early part (first 2 wk) of the 14-wk incubation (Fig. 1). However, although the initial rate of N release may differ between air-dried and field-moist soil, the difference in the total amount of N mineralized in 14 wk is likely to be relatively small (Beauchamp et al., 1986). The flush of mineralization in the early phase of the incubation may be partly due to mineralization of microbial biomass killed during drying and re-wetting and partly due to decomposition of non-biomass organic matter released when the soil was re-wetted (Wu and Brookes, 2005). The low ratio of mineralized C to mineralized N in the early weeks of the incubation (relative to the C to N ratio of soil organic matter) suggests that protein-rich microbial tissue made a significant contribution to the initial flush of mineralization. Greater kill-off of soil microbes would be expected when pastoral soils are dried and re-wetted because of their large

Table 4. Correlation coefficients (*r*) between indices of N mineralization potential (*n* = 130).

Parameter	Total C	Total N	POMt–C	POM- N	AMN 40°C	AMN 25°C	$NaHCO_3$–C	CO_2 burst	CWEON	CWEC	HWEC	HWEON	HW SUVA	HW NH_4–N
Total C	1.00‡													
Total N	0.97	1.00												
POM-C	0.90	0.89	1.00											
POM-N	0.89	0.90	0.99	1.00										
AMN 40°C	0.70	0.73	0.70	0.68	1.00									
AMN 25°C	0.61	0.63	0.58	0.56	0.90	1.00								
$NaHCO_3$–C	0.78	0.78	0.73	0.73	0.73	0.72	1.00							
CO_2 burst	0.59	0.60	0.54	0.51	0.87	0.89	0.75	1.00						
CWEON	0.47	0.51	0.46	0.44	0.79	0.76	0.65	0.81	1.00					
CWEC	0.66	0.67	0.62	0.59	0.86	0.85	0.83	0.88	0.91	1.00				
HWEC	0.76	0.76	0.69	0.67	0.86	0.85	0.86	0.89	0.83	0.93	1.00			
HWEON	0.69	0.70	0.63	0.61	0.87	0.89	0.81	0.92	0.87	0.92	0.98	1.00		
HW SUVA	0.54	-0.53	-0.45	-0.44	-0.43	-0.46	-0.52	-0.45	-0.07	-0.32	-0.46	-0.42	1.00	
HW NH_4–N	0.91	0.92	0.87	0.87	0.84	0.76	0.82	0.77	0.66	0.79	0.88	0.84	-0.54	1.00
Min. pot.	0.66	0.69	0.62	0.60	0.86	0.90	0.81	0.92	0.79	0.87	0.92	0.94	-0.47	0.81

† Abbreviations: POM, particulate organic matter; AMN, anaerobically mineralizable N; CWEON, cold water extractable organic N; CWEC, cold water extractable C; HWEC, hot water extractable C; HWEON, hot water extractable organic N; HW SUVA, specific ultraviolet absorbance measured in hot water extract; HW NH_4–N, ammonium N generated during hot water extraction; Min. pot., N mineralization potential (N mineralization in 14 week incubation).

‡ Correlation coefficients [3]0.17 were statistically significant at the 0.05 probability level; Correlation coefficients [3]0.23 were statistically significant at the 0.01 probability level.

microbial populations (Haynes, 2000) and this may partly explain why the ratio of mineralized C to mineralized N was lower in pastoral than in arable soils.

The degree of physical disturbance (fineness of sieving) may also influence the results if protected organic matter becomes accessible to soil microorganisms due to aggregate disruption (Beare et al., 1994). However, our previous research showed that mineralization in coarsely-sieved soil (soil screened through a 4-mm sieve, as in this study) did not differ from that in intact soil cores (Curtin et al., 2014). We conclude that the total quantity of N mineralized from re-wetted, air-dried soil over 14 wk gives a robust measure of the soils' potential to mineralize N. These mineralization values, representing a broad range of land uses and soil types, provide a solid benchmark against which to evaluate the performance of the selected rapid assays and tests.

The very wide range of N mineralization potentials measured in the 130 soils included in the study was, to a considerable extent, a reflection of differences in land use history, with values for pasture soils being, on average, 2.3 times those for soils with a history of arable or vegetable cropping. On an area basis, the mineralization potential of pasture soils amounted to 360 ± 111 kg N ha^{-1} vs. 160 ± 44 kg ha^{-1} in cropped soils (values estimated assuming a soil bulk density of 1.2 g cm^{-3}). These results confirm that New Zealand soils may supply large (but variable) quantities of N, and therefore proper allowance for mineralized N is essential for accurate prediction of N fertilizer requirements to meet plant demand while minimizing the risk of N losses to the wider environment. There was no evidence that the N mineralization potential of pastoral soils differed depending on the specific land use (dairy, sheep/beef). Similarly, type of cropping (arable or vegetable cropping) did not affect N mineralization potential. However, it should be noted that the soil sampling design did not allow for a balanced representation of land uses in each soil order, so the strength of our conclusions about land use effects is limited by these constraints.

As field conditions (particularly temperature and soil moisture) will often be less favorable for microbial activity than those in our incubation, the N mineralization potential measured over 14 wk may overestimate the amount of N supplied to plants during a growing season. Parfitt et al. (2005) showed that annual N

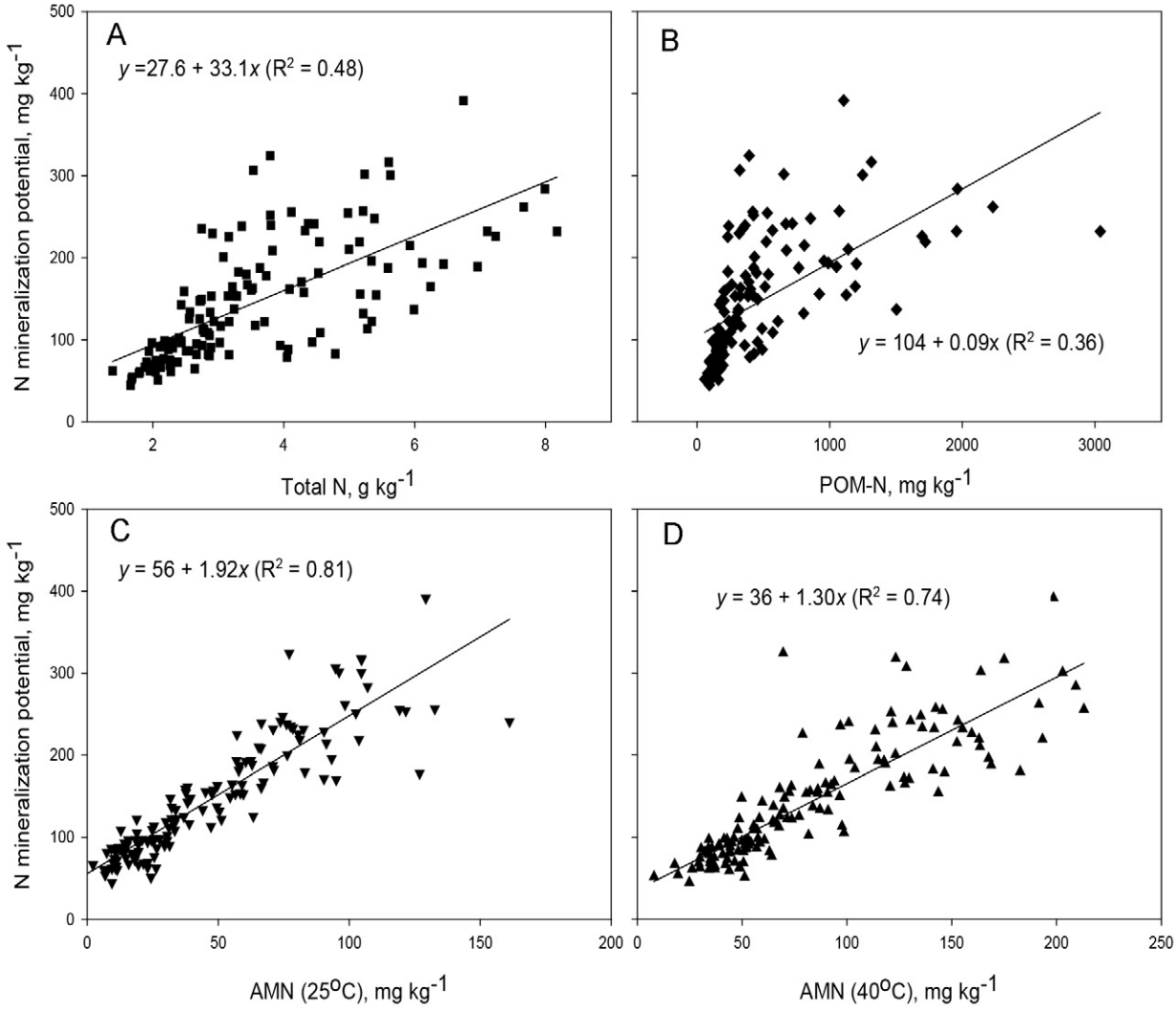

Fig. 2. Relationship of total N (A), particulate organic (POM)-N (B), and anaerobically mineralizable N (AMN) measured at 25°C (C) and 40°C (D) with N mineralization potential (N mineralized in a 14 wk aerobic incubation at 25°C).

uptake by unfertilized grass-clover pastures (41 to 304 kg ha^{-1}) in the North Island of New Zealand corresponded with amounts mineralized in 8 wk at 25°C (soil incubated at 60% of water holding capacity). However, in that study pasture growth (and N uptake) was severely restricted by drought during a very dry summer (January to April) period. This limited plant growth and therefore N uptake.

The indicators that were evaluated varied in their ability to predict mineralization potential. Although recent studies (using pasture soils) showed that total soil N can be a good predictor of N mineralization (McDonald et al., 2014; Shepherd et al., 2015), this was not borne out by our study. Two factors contributed to the poor relationship between total soil N and N mineralization potential in our study. First, the pastoral soils in this study mineralized a larger proportion of total soil N than cropping soils (Table 2), consistent with many studies showing that pastoral soils contain a relatively large component of labile organic N (e.g., Curtin and McCallum, 2004; Haynes, 2000). Second, on average, allophanic soils mineralized a smaller proportion of total soil N than sedimentary soils. The latter observation is in line with evidence of lower biodegradability of organic matter in allophanic soils because of the stabilizing influence of allophane and associated minerals such as ferrihydrite (Parfitt et al., 1997).

As with N mineralization potential, POM-N was more abundant in pastoral than in cropped soils (Table 1). Even so, the correspondence between POM-N and mineralization potential was poor overall. As the N concentration in POM is low relative to C (C to N ratio 14.1 ± 1.8), some immobilization of N may have occurred during the 14-wk incubation, obscuring the contribution of POM to (net) N mineralization. This explanation is consistent with Bimüller et al. (2014) who showed that net N mineralization in POM isolated from forest soil was small compared with that of organic matter associated with the clay and silt fractions. The importance of the POM C to N ratio was demonstrated in a study in which POM material with a range of N concentrations (C to N ratio of 12.4 to 14.9) was added to soil in a 28-d incubation (St. Luce et al., 2016). A small net mineralization of N was observed where POM with a low C to N ratio was added but net immobilization occurred in soil treated with POM with high C to N ratio.

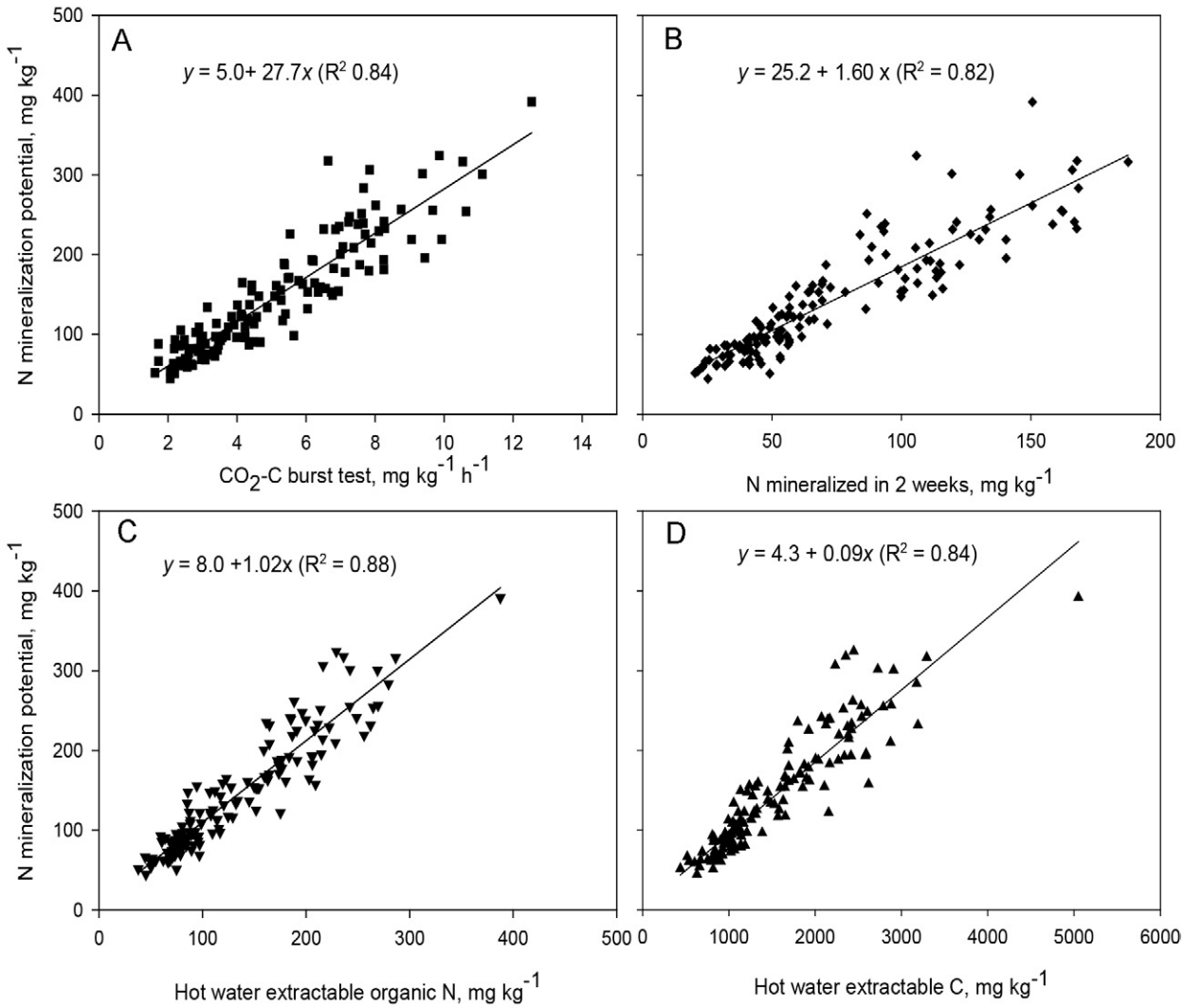

Fig. 3. Relationship of CO_2 burst test (A), N mineralized in first 2 wk of aerobic incubation (B), and hot water extractable organic N (C), and hot water extractable C (D) with N mineralization potential measured in a 14 wk aerobic incubation at 25°C.

The allophanic soils had low N mineralization potentials relative to their POM contents, but the reason was not identified. It is possible that POM was overestimated because the very stable aggregates in these soils were not completely dispersed, resulting in "contamination" of POM with clay- and silt-sized material. However, even when the allophanic soils were excluded and the analysis confined to sedimentary soils, the relationship between POM-N and mineralization potential remained relatively weak ($r = 0.72$).

The CO_2 burst test, which is considered a general indicator of soil biological activity (Haney et al., 2008), was more closely related with N mineralization potential than was AMN (40°C), the current soil N test in New Zealand. The burst test, which is rapid and easy to perform, appears to have significant merit as a soil N test. A similar conclusion was reached by Franzluebbers (2016), based on results from ongoing greenhouse and field trials in the southern United States. The CO_2 burst test was sensitive to land use history (pasture vs. cropping; Table 2) and the relationship with mineralization potential was similar for sedimentary and allophanic soils.

A plot of AMN at 40°C vs. mineralization potential showed there was considerable scatter in the data, suggesting that fertilizer N recommendations based on the AMN test may not be very reliable. The better performance of the C-based burst test compared with AMN, a test that is specific for N, might be because the CO_2 burst was measured at the same temperature (25°C) and moisture and/or aeration conditions as N mineralization potential. However, reducing the AMN measurement temperature from 40 to 25°C resulted in only a marginal improvement in the relationship with mineralization potential (Fig. 2).

Assuming the temperature response of mineralization is described by Q_{10} of 2 (Kirschbaum, 1995), AMN at 25°C would be expected to be ~35% of that at 40°C. For cropped soils, AMN at 25°C averaged 39 ± 13% of that at 40°C, consistent with a Q_{10} of ~2. In pastoral soils, AMN was less responsive to temperature; the 25°C value averaged 62 ± 14% of that at 40°C (Q_{10} ~1.4). The reason for the low temperature dependence of AMN in pastoral soils is not known. The slope of the relationship between mineralization potential and AMN increased when AMN measurement temperature was decreased to 25°C (Fig. 2) but the increase was relatively small because of the low temperature dependence of AMN in pastoral soils (soils with generally high AMN). The other consequence of low temperature dependence of AMN in pastoral soils is that the intercept in the mineralization potential vs. AMN relationship increased when AMN measurement temperature is reduced from 40 to 25°C (Fig. 2). Based on the present results, there does not seem to be a strong case to lower the AMN measurement temperature from 40°C. Although the AMN test (40°C) has been used in New Zealand for many years, it has not been field calibrated for either crops or pastures. The present results suggest that mineralization potential can be approximated by multiplying the AMN value by a factor of 1.5; however, this factor may vary considerably for individual soils.

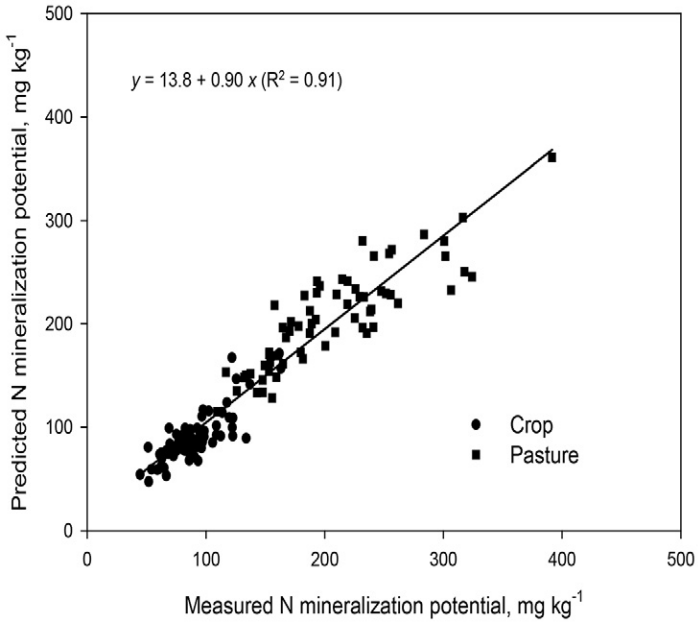

Fig. 4. Nitrogen mineralization potential predicted using a model that included hot water extractable organic N, SUVA, and land use versus mineralization potential measured in a 14 wk incubation at 25°C.

The N released from air-dry soil during a short (2-wk) aerobic incubation may provide a more reliable prediction of N mineralization potential than AMN. This 2-wk value, which is strongly influenced by the size of the mineralization flush on re-wetting, was moderately well correlated to mineralization potential (Fig. 3). However, the relationship was not as close as that between mineralization potential and hot water extractable organic matter, which can be easily and quickly measured, and is more suited to routine use in commercial laboratories.

Of the three extraction methods that were evaluated, the hot water extraction method was, by far, the most promising predictor of N mineralization potential. An underpinning rationale for the use of extraction methods is that the extracted organic matter is a dominant or, at least, important substrate for soil microorganisms. There is compelling evidence that organic matter must be in dissolved form to diffuse to, and be transported across, microbial cell membranes (Schimel and Bennett, 2004). Water is the natural solvent in soils and an extraction at room temperature (cold water) possibly provides a measure of the immediately available organic N. This small pool of organic N (7 to 51 mg kg^{-1} in our soils), which is likely to be metabolized within days, is "buffered" so that, when mineralized, additional soluble organic matter is released into the soil solution (Curtin et al., 2012). The utility of hot water as a predictor of mineralization potential may be because it measures both the immediately available organic matter and the capacity of soil to release organic matter over time. Our results show the quantity of organic N extracted in hot water (single extract, without the preliminary cold water extraction) was similar to that mineralized during the 14-wk aerobic incubation at 25°C. An important factor in relation to the efficacy of hot water as an organic matter extractant is that disturbance of the chemical controls on organic matter solubility

is minimized. In contrast, the other chemical extractant used in this study, 0.01 mol L^{-1} $NaHCO_3$, will raise soil pH and displace exchangeable cations, and both of these chemical changes may affect organic matter solubility (Curtin et al., 2016).

The sensitivity of HWEON to management history and soil type (larger proportion of total soil N extracted from pastoral vs. cropped soils; and from sedimentary vs. allophanic soils) mirrored the influence of these factors on N mineralization potential. An ability to appropriately reflect the influence of land use history on the supply of plant available N is a universal prerequisite for a soil N test. In New Zealand, where volcanic soils are common, sensitivity to soil type (e.g., allophanic vs. sedimentary) is also important. Hot water extractable N meets these requirements well and, based on the results that have been presented, it appears to be a test that could be applied to agricultural soils regardless of land use or parent material. On the assumption that similar mechanisms operate to regulate the supply and microbial breakdown of mineralizable organic matter in soils around the world, there is good reason to believe that the HWEON test would be a useful predictor of N mineralization potential in sedimentary and allophanic soils globally.

It has been suggested that the accuracy of mineralizable N testing might be improved by a change from the "single test" approach to one based on a combination of N tests (Ros et al., 2011b). In practice, it is unlikely that any more than two tests would be used and, therefore, we combined HWEON (as best single predictor) with either total N, AMN (40°C), POM-N, or the CO_2 burst test. However, none of these combinations significantly improved the prediction of mineralization potential compared with HWEON alone.

Although hot water extractable organic matter consists primarily of carbohydrates and N-containing compounds (amino-N and amides) that are readily biodegradable, part of it may be relatively recalcitrant (Chantigny et al., 2014; Gregorich et al., 2003; Leinweber et al., 1995). Partitioning it into labile and recalcitrant components might improve the sensitivity of hot water extractable N as a predictor of N mineralization potential, though this would add to the analytical costs. Measurement of UV absorbance may provide a rapid and cheap estimate of the proportion of aromatic (lower biodegradability) compounds in the water soluble fraction of organic matter (Dilling and Kaiser, 2002; Weishaar et al., 2003). The SUVA data suggested that the quality of hot water extractable organic matter differed between pastoral and cropped soils, i.e., higher SUVA values indicated that extractable organic matter was more aromatic in cropped soils. Consistent with this, there was a significant negative correlation between SUVA and mineralization potential (Table 4). Inclusion of SUVA in a multivariate regression model with HWEON resulted in a small, but significant, improvement in the relationship with N mineralization potential.

In keeping with previous work (Chantigny et al., 2010; Curtin et al., 2006), a significant amount of ammonium N (6 to 76 mg N kg^{-1}) was released during the 16-h hot water extraction. This ammonium N was more closely related to total soil N than to HWEON, possibly suggesting that its source was the water-insoluble fraction of organic matter rather than the relatively small hot water extractable fraction. The ammonium N released during hot water extraction is likely of similar origin to the ammonium released on treatment with hot 2 mol L^{-1} KCl, which has been evaluated as a soil N test in its own right (Gianello and Bremner, 1986; Sharifi et al., 2007). The ammonium N released by hot water was not as well correlated with mineralization potential as the organic N (R^2 of 0.66 vs. 0.88). We therefore recommend that hot water organic N (ammonium N excluded) be used to assess N mineralization potential.

Only in a few recent studies has hot water extractable organic matter been considered as an indicator of N mineralization potential (Ros et al., 2011a, 2011b; Thomas et al., 2015) so few comparisons can be made with published work. Ros et al. (2011a) estimated that N mineralized in Dutch soils during an 18-wk incubation at 20°C was equivalent to about 70% of hot water extractable N. This seems generally consistent with our finding that HWEON was quantitatively similar to N mineralized in 14 wk at 25°C. It is tempting to conclude on the basis of these results that there is a direct causal link between HWEON and mineralizable N (i.e., the organic matter extracted in hot water is the actual substrate utilized during incubation) but that is not possible based on a statistical relationship. Further work is necessary to provide a mechanistic explanation for close correspondence between hot water extractable organic matter and N mineralization potential.

CONCLUSIONS

In this study, we evaluated the ability of a range of "quick tests" to predict N mineralization potential, as measured in a 14-wk aerobic incubation at 25°C. We identified hot water extractable N and the CO_2 burst test as being superior to the current New Zealand test, AMN. These two tests were strongly correlated (R^2 = 0.84) possibly because dissolved organic matter is a key driver of the burst of CO_2 that results from re-wetting of dry soil. However, the hot water test was more closely correlated with N mineralization potential and has the advantage of being easier to implement in a commercial laboratory setting. Our laboratory assessments suggest that the hot water test is a robust test that can provide reliable predictions of soil N supplying capacity across a wide range of land uses and soil types. As such, it is a tool that can support better decisions with respect to N fertilizer management.

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