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LEGUME RESIDUE AND SOIL WATER EFFECTS ON DENITRIFICATION IN SOILS OF DIFFERENT TEXTURES

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Summary—Legume cover crops commonly used to supply additional N and reduce potential for over-winter N leaching losses may also influence denitrification depending upon soil water status and soil type. Interrelationships between incorporated hairy vetch (*Vicia villosa*) residue and soil water status on denitrification in coarse, medium and fine textured soils were investigated in the laboratory. Repacked soil cores were incubated, 10, 20 and 30 d with and without acetylene (C_2H_2). Denitrification losses were 20–200 μg N kg^{-1} from each soil when 60% of the soil pore space was filled with water and increased to from 14.0 to 18.6 mg N kg^{-1} at 90% water-filled-pore space (WFPS). Incorporation of vetch residue (2.5 g kg^{-1}) greatly stimulated denitrification (51.1–99.5 mg N kg^{-1}), probably due to greater availability of organic C as indicated by higher CO_2 emissions. The major denitrification losses occurred during the first 10 days and more so in residue-amended soils. The supply of C from incorporated legume crop residue was a major factor influencing denitrification especially when soil wetness restricted aeration and adequate nitrate was present. At similar water contents, rates of denitrification differed greatly in soils of varying texture, but when varying water holding capacity and bulk density were accounted for using WFPS, all soils behaved very similarly. Use of WFPS as an index of aeration status enabled identification that differences in denitrification losses in vetch-amended soils of varying texture resulted in part from varying capacity to supply NO_3^- and metabolize organic matter. These results illustrate the utility of WFPS, compared with soil water content, and its reliability as an indicator of reduced aeration dependent denitrification for soils of varying texture.

INTRODUCTION

Increasing cost of inorganic fertilizers and decreasing soil productivity has prompted interest in the use of annual legumes as alternative N sources. Use of legume cover crops in conservation tillage reduces erosion potential, improves surface and ground water quality (due to nutrient uptake and storage), increases organic matter content, enhances infiltration, improves soil structure, weed control, and enhances soil N availability (Langdale *et al.*, 1991; Sharpley and Smith, 1991; Doran and Smith, 1991). The potential for legume crops to conserve soil and water resources has been well documented over diverse cropping environments in the U.S.A. (Power, 1987), as an alternative to summer fallow in Canadian Prairies (Aulakh *et al.*, 1983), and as green-manures in various cropping systems in Asia (Abrol and Palaniappen, 1988; Yadvinder-Singh *et al.*, 1991). Green legume crop residues, at early growth period decompose rapidly when incorporated into soil and release not only mineral N but also supply considerable organic C to soil microorganisms. Thus, the addition of legume residues could increase the losses of N through denitrification if soil is relatively wet or anaerobic (Stefanson, 1972; John *et al.*, 1989; Andren *et al.*, 1990; Aulakh *et al.*, 1991a).

Soil texture may influence the denitrification process in several ways. Exposed soil surfaces provide attachment sites for microbial cells and negatively-charged soil colloids may concentrate nutrients on the colloid surface (Focht and Verstraete, 1977). Higher rates of denitrification have been reported in finer-textured soils (Lund *et al.*, 1974; Chaterpaul *et al.*, 1980). This, however, may not be due to a direct effect of the clay on the microorganisms since van der Staay and Focht (1977) were unable to show any differences in denitrification rates between clays of different particle size after removal of indigenous organic matter and subsequent addition of substrate and inoculum. The effect of soil texture on denitrification likely results from natural differences in capacity of soils to supply NO_3^- and C, and also due to physical variations in soil structure, pore size, aggregation and water infiltration rates that affect aeration, water holding-absorption capacity and microenvironment. In the field study of Chaterpaul *et al.* (1980), increased denitrification associated with the degree of fineness of soil texture was directly related to soil water content.

Potential for denitrification in soils is a complex interaction between aeration, nitrate availability, carbon substrate availability and other intrinsic "soil factors" (Firestone, 1982). It is relatively well-known that the absence of O_2 or reduced O_2 availability is required for both the synthesis and activity of denitrification enzymes. Quantification of O_2 availability and related rates of denitrification in soils, however, is complicated by dynamic relationships between

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Table 1. Soil characteristics, experimental bulk density, initial soil nitrate and ammonium-N concentrations, and soil water content at 60 and 90% WFPS for repacked cores of three surface soils

Characteristics	Soil		
	Houston Black	Gurdaspur	Tolewal
Texture	Silty clay	Silt loam	Sandy loam
Taxonomy	Udic pellustert	Udic haplustalf	Typic ustipsamment
Location	Temple, Texas (U.S.A.)	Gurdaspur, Punjab (India)	Ludhiana, Punjab (India)
Mechanical analysis			
Sand (%)	13	29	71
Silt (%)	43	50	18
Clay (%)	44	21	11
pH	7.2	7.2	7.8
Organic C (g kg^{-1})	11.0	5.7	4.0
Total N (g kg^{-1})	1.2	0.7	0.5
NO_3^--N (mg kg^{-1})*	164	111	101
NH_4^+-N (mg kg^{-1})	5.6	2.2	1.0
Bulk density (Mg m^{-3})	1.0	1.3	1.5
At 60% WFPS			
Volumetric H_2O (cm^3 cm^{-3})	0.37	0.30	0.26
Gravimetric H_2O (g g^{-1})	0.37	0.23	0.17
At 90% WFPS			
Volumetric H_2O (cm^3 cm^{-3})	0.56	0.46	0.39
Gravimetric H_2O (g g^{-1})	0.56	0.35	0.26

*Nitrate N content at the beginning of the incubation including amount of nitrate N added.

aeration potential (O_2 flux) and microbial oxygen use (Greenwood, 1975). While water content, matric potential and water-holding capacity all serve as relative predictors of microbial activity in soil, the expression of water content as a volumetric percentage of soil pore space also encompasses the role of water as a barrier to O_2 diffusion and as such is a better measure of factors regulating aeration-dependent microbial processes (Sommers *et al.*, 1981).

In most soil denitrification research, soil water content is usually expressed on a gravimetric or volumetric basis or on soils' water-holding capacity without consideration of differences in density or porosity for soils of varying textures. Therefore, it has been difficult to obtain conclusive results on the effects of soil texture. To overcome problems of varying soil texture and bulk-density, variable factors such as water-filled pore space (WFPS), or alternatively air-filled pore space (100-WFPS), which incorporate both soil water content and bulk density, may be better indices of aeration dependent biological processes, such as denitrification, than soil water content (Doran *et al.*, 1990; Skopp *et al.*, 1990). However, when soil water contents approach or exceed field capacity, the percentage of soil pore space filled with air or water are considered better indicators of aerobic vs anaerobic microbial activity than either water content, matric tension, or water potential (Miller and Johnson, 1964; Sommers *et al.*, 1981).

Therefore, the present study was conducted to investigate the effects of hairy vetch (*Vicia villosa*) residue addition and soil water status (comparing water content with WFPS) on N losses via denitrification from soils of widely varying texture.

MATERIALS AND METHODS

Soils used in the study were collected from the A horizon of benchmark sites, one in the United States and two in India. The soils varied in texture, organic C, total N, and mineral N content (Table 1). Air-dried soils were crushed to pass through a 2 mm sieve and thoroughly mixed. To re-establish soil biological activity after collection and storage, all soils were conditioned before experimentation by moistening bulk soil samples to 40% water-filled pore space (WFPS) and kept for 2 weeks in a growth chamber at 25°C (Doran *et al.*, 1990). Water-filled pore space, synonymous with relative saturation, was calculated as follows: WFPS = [(gravimetric water content × soil bulk density)/total soil porosity] where soil porosity = [1 − (soil bulk density/2.65)] and 2.65 was the assumed particle density of soil. Experimental treatments for each soil consisted of two soil water contents equivalent to 60 and 90% WFPS and two rates of hairy vetch residue (0 and 2.5 mg dry matter kg^{-1} soil). Hairy vetch (*Vicia villosa* cv. Madison) residue was collected green from a 60 day old crop which had a C:N ratio of 8 and a N content of 5.7% (on dry weight basis). Each treatment was triplicated in 3 groups (or batches) to incubate for various periods (Table 2). Repacked soil cores were prepared by placing moist conditioned soil (60 g on oven dry basis) with or without incorporated hairy vetch residue

Table 2. Description of total incubation period and time of C_2H_2 addition, gas sampling and KCl extraction

Batch No.	Total incubation period (days)	Sealed with C_2H_2 on day (s)	Gas sampling on day (s)	Extract with KCl on day
1	10	0, 1, 2, 3, 6	1, 2, 3, 6, 10	10
2	20	10, 11, 13, 16	11, 13, 16, 20	20
3	30	20, 21, 24	21, 24, 30	30

in 100 ml plastic vials and hand compacting to bulk densities representative of natural reconsolidation values for each soil.

Gurdaspur SiL and Tolewal SL had much lower soil nitrate N concentration than the Houston Black SiC and therefore KNO_3 was added in solution to raise their initial nitrate content (Table 1). Deionized water was added to obtain required WFPS and then individual vials were placed in 0.95-litre glass jars sealed with screw-cap lids in which a serum stopper had been fitted for gas sampling. Jars of Batch 1 were flushed with atmospheric air and 10% (V/V) acetylene (C_2H_2) was added at 0 d. All jars were kept at 25°C. Periodically, duplicate gas samples (1-ml) of the headspace of each C_2H_2 amended jar were taken for N_2O and CO_2 analysis, then jars were flushed with atmospheric air, fresh C_2H_2 was added, and the jars were resealed. Jars of all batches were flushed and resealed in a manner similar to those receiving C_2H_2. After the last gas sampling, the soil cores were broken by shaking jars in each batch (Table 2) on a mechanical shaker in a horizontal position for 60 min and then gas samples were again taken to measure N_2O and CO_2 entrapped in the soil (Aulakh and Doran, 1990).

Nitrous oxide content in gas samples was determined using a Varian 3700 gas chromatograph equipped with a ^{63}Ni electron capture detector at an operating temperature of 350°C with $Ar:CH_4$ (95:5 ratio) as a carrier gas at a flow rate of 60 ml min^{-1}. A 1.8 m column of Porapak Q (50/80 mesh) was used for separation of N_2O, water vapor was removed with a precolumn of 8 mesh $CaCl_2$. CO_2 concentrations were determined using a thermal conductivity detector at an operating temperature of 110°C, a column oven temperature of 60°C, and He as a carrier gas at a flow rate of 55 ml min^{-1}. A 1.8 m column of Chromosorb 106 with a $CaCl_2$ precolumn was used for separation and detection of CO_2. Data were corrected for the solubility of N_2O and CO_2

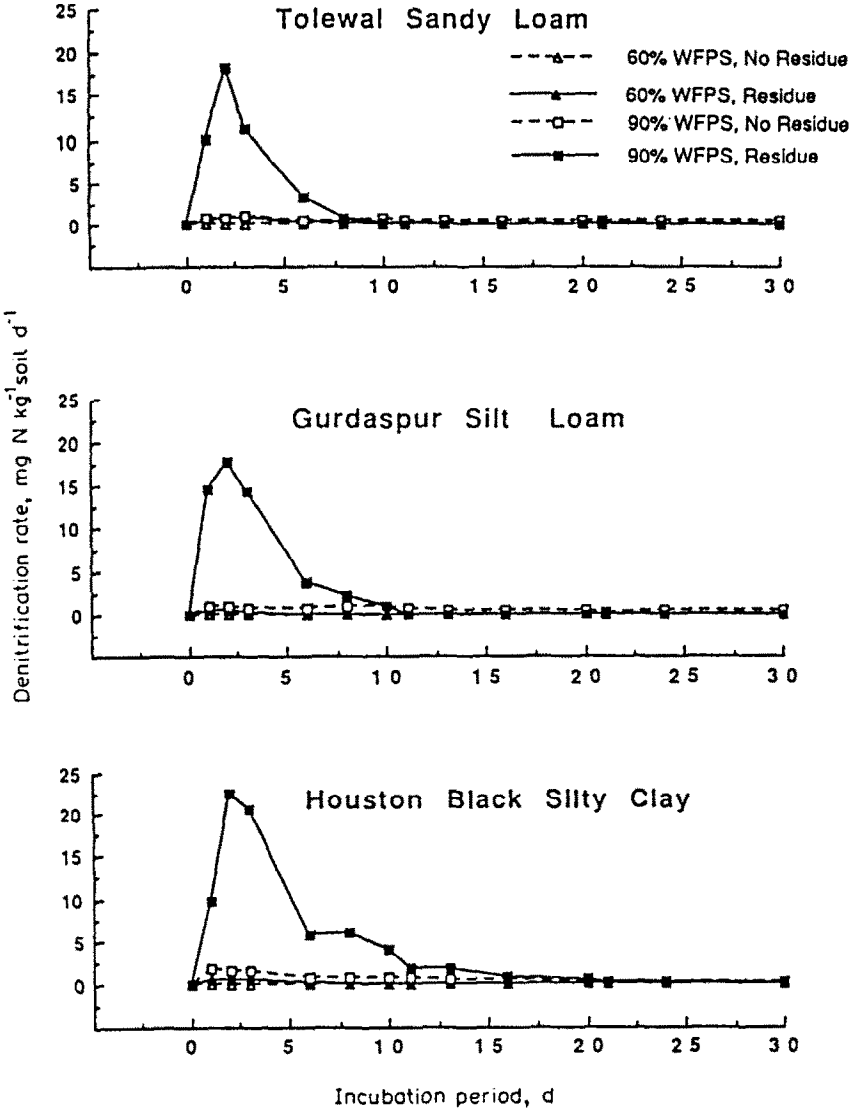

Fig. 1. Rate of denitrification in three soils of varying texture incubated at 60 and 90% WFPS with or without hairy vetch crop residue. Actual days of gas sampling given in Table 2.

Table 3. Cumulative denitrification losses from three soils of varying texture, incubated at 60 and 90% WFPS without or with incorporated hairy vetch residue

Soil	60% WFPS				90% WFPS			
	0–10 d	11–20 d	21–30 d	Total	0–10 d	11–20 d	21–30 d	Total
	mg N kg^{-1}							
	Without hairy vetch residue							
Tolewal SL	0.00e*	0.03c	0.00c	0.04e	6.06f	3.78c	4.18b	14.02e
Gurdaspur SiL	0.16d	0.01c	0.01c	0.18d	8.33e	4.77b	5.49a	18.59d
Houston Black SiC	0.11d	0.01c	0.01c	0.03e	8.87d	3.54c	2.17c	14.58e
	With hairy vetch residue							
Tolewal SL	3.10a	0.18a	0.02c	3.30a	50.83c	0.06d	0.25e	51.14c
Gurdaspur SiL	1.32c	0.12b	0.20a	1.64c	64.05b	0.02d	0.01f	64.08b
Houston Black SiC	1.76b	0.13b	0.08b	1.97b	89.39a	9.33a	0.81d	99.53a

*Values in each column followed by same letter do not differ significantly ($P \geq 0.05$).

in soil water (Moraghan and Buresh, 1977). At the termination of the incubation period of each batch, the soil of each vial was mixed well and a 10 g sample was extracted with 1 M KCl (30 min shaking) followed by filtration and determination of NO_3^--N and NO_4^+-N by automated colorimetric analysis using cadmium reduction and indophenol blue (Keeney and Nelson, 1982).

Experimental design was a factorial completely-randomized design with three replications. The factors of the model were soil texture, crop residue, WFPS and their interaction. Statistical analysis was made on log-transformed data [$\log_{10}(x + 1)$] for N_2O-N and CO_2-C in order to satisfy the assumption of variance homogeneity. Means were separated by the Least Significant Difference. The employed procedures are reported in the Statistical Analysis System (SAS Institute, 1985).

RESULTS AND DISCUSSION

The losses of N due to denitrification were negligible from Tolewal SL, Gurdaspur SiL, and Houston Black SiC at 60% WFPS with rates ranging between 0–30 μg N kg^{-1} d^{-1} (Fig. 1). When water content was increased to 90% WFPS, the rates increased substantially during the initial 10 days without crop residue (Table 3). Incorporation of 2.5 g kg^{-1} of young hairy vetch residue increased the rate of denitrification several fold with maximum rates of 17.8–22.4 mg N kg^{-1} d^{-1} occurring on the second day of incubation in each soil and decreased rapidly thereafter. In other treatments (60% WFPS with or without residue, 90% WFPS without residue) denitrification rates were lower but the highest rates also occurred on the second day. In soils at 90% WFPS without residue, the rate of N loss was low and remained relatively constant throughout the study (Fig. 1).

Total N losses via denitrification without residue during the 30-d incubation were lowest (30–200 μg N kg^{-1}) at 60% WFPS and greatest (14.0–18.6mg N kg^{-1}) at 90% WFPS (Table 3). With incorporated hairy vetch residue, the losses ranged from 1.6 to 3.3 mg N kg^{-1} at 60% WFPS and from 51.1 to 99.5 mg N kg^{-1} at 90% WFPS. In residue-amended soils at 90% WFPS, the proportion of losses that occurred during the initial 10 d ranged from 80 to 99% of the total N losses in the three soils. In soils incubated without residue at 90% WFPS, N losses ranged from 43 to 61% in the initial 10 d, 24–27% in the 11–20 d period, and 15–30% in the 21–30 d period (Table 3).

In residue-amended soils, at 90% WFPS soil NO_3^--N decreased rapidly in all soils from denitrification and immobilization (Table 1); residual NO_3^--N ranged from 0 to 23 mg N kg^{-1} at 10 d and was undetectable thereafter (Table 4). Bijay-Singh *et al.* (1988) demonstrated an association between denitrification potential in soils and the amount of C susceptible to mineralization under aerobic or anaerobic conditions. In the present study, patterns of C mineralization in soils at 60 and 90% WFPS with or without hairy vetch residue (Fig. 2) were similar to those for N_2O emissions at 90% WFPS (Fig. 1) during the initial 10 d, with a peak in both denitrification and CO_2 production rates by day 2. During the later part of study (11–20 d), C mineralization continued at relatively high rates (17.2–28.7 mg CO_2-C kg^{-1} d^{-1}) in residue-amended soils at 90% WFPS but rates of denitrification rates decreased, presumably

Table 4. Nitrate-N content in three soils of varying texture, incubated at 60 and 90% WFPS without or with incorporated hairy vetch residue (soils exposed to C_2H_2 for each 10 d incubation period only)*

Soil	60% WFPS			90% WFPS		
	10 d	20 d	30 d	10 d	20 d	30 d
	mg N kg^{-1}					
	Without hairy vetch residue					
Tolewal SL	63b†	75e	76f	48b	51b	45b
Gurdaspur SiL	65b	98b	102e	61b	47b	36c
Houston Black SiC	167a	183b	179b	134a	131a	119a
	With hairy vetch residue					
Tolewal SL	58b	97b	118d	0c	0c	0d
Gurdaspur SiL	69b	143c	156c	0c	0c	0d
Houston Black SiC	170a	233a	250a	23c	0c	0d

*Soil NO_3^--N content at 0 d was 101, 111 and 164 mg N kg^{-1} for Tolewal SL, Gurdaspur SiL and Houston Black SiC, respectively.

†Values in each column followed by same letter do not differ significantly ($P \geq 0.05$).

due to exhaustion of NO_3^--N (Table 4). In the case of soils without added residue at 90% WFPS (where NO_3^--N remained high), the relatively low but almost constant rates of denitrification throughout the study may have been limited by continuous low supply of organic C to denitrifying organisms through the mineralization of native soil organic matter. Therefore the supply of available C, whether from native organic matter or from the incorporated crop residue, was a major factor influencing denitrification, especially when soil aeration was restricted and adequate amounts of NO_3^- were present. These results strongly suggest that supply of C will determine the rate of denitrification in nearly saturated soils until the NO_3^- supply is exhausted (Rice *et al.*, 1987; Aulakh *et al.*, 1991b).

Although the rates of denitrification from residue-amended soils at 60% WFPS were not as pronounced as at 90% WFPS, the influence of incorporated crop residue was evident in each soil. Total N losses from residue-amended soils at 60% WFPS were 1–2 orders of magnitude higher than those from soils without residue (Table 3). These results confirm that decomposition of incorporated low C:N ratio plant residues in soils apparently increases denitrification through increased microbial activity (as revealed by soil CO_2 production), and respiratory O_2 consumption from metabolically-active soil microsites as shown earlier by others (Greenwood, 1975; Aulakh and Rennie, 1987; Rice *et al.*, 1988). These results support the observations made by Parkin (1987) on soil microsites (hot spots) and Doran (unpublished, reported in Aulakh *et al.*, 1991a) in a field study involving crop residue management who observed localized habitats of greater bioactivity created conditions favorable for denitrification even at WFPS of 60% or below.

Lund *et al.* (1974) reported greater rates of denitrification in finer-textured soils. Later studies indicated that the influence of soil texture on denitrification was

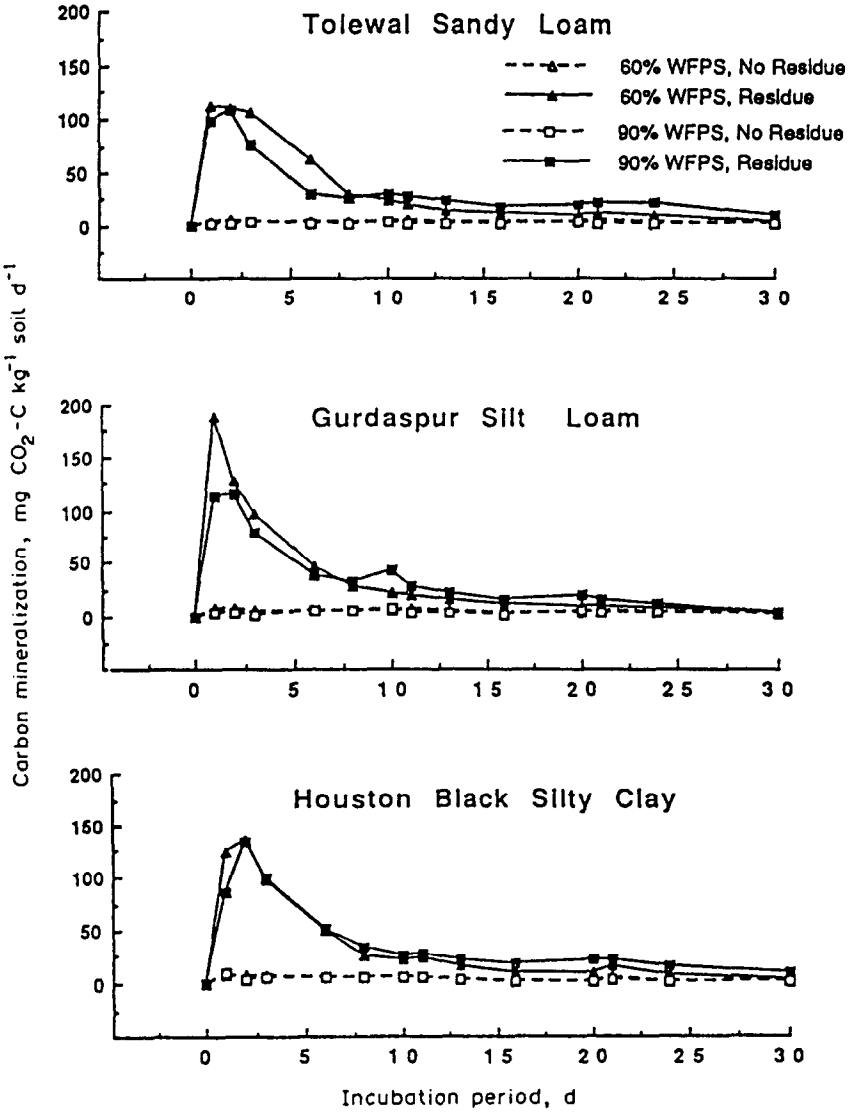

Fig. 2. Rate of carbon mineralization in three soils of varying texture incubated at 60 and 90% WFPS with or without hairy vetch crop residue. Actual days of gas sampling given in Table 2.

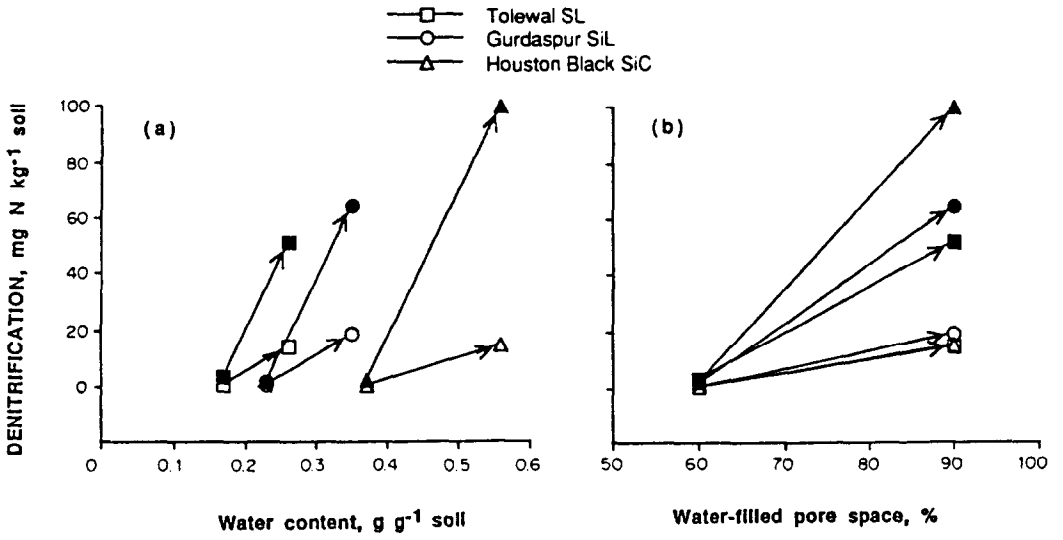

Fig. 3. Relationship of total denitrification losses with (a) soil water content and (b) water-filled pore space in three soils of varying soil texture incubated with (solid symbols) and without hairy vetch residue (open symbols).

not a direct effect of clay on microorganisms (van der Staay and Focht, 1977) but was related to soil water content (Chaterpaul *et al.*, 1980), which influences aeration in soils. Soil water contents in those studies were expressed on a gravimetric or volumetric basis. However, the same soil water content in texturally different soils provides very different conditions of soil aeration and associated denitrification activity. For example, in our study, a water content of 37% (gravimetric) resulted in 60% WFPS (40% air-filled pore space) or aerobic conditions in Houston Black SiC but in almost saturated conditions (90% WFPS) in Gurdaspur SiL and consequently several-fold higher denitrification rates [Fig. 3(a)]. Similarly 37–39% volumetric water content allowed relatively more air-filled porosity in clay (60% WFPS) but saturated conditions in the sandy loam soil and resulted in greatly different rates of denitrification [Fig. 3(a)]. To integrate the effects of varying soil texture, bulk density and water content with varying tillage management Linn and Doran (1984) suggested that a measure such as a water-filled pore space was a reliable, and relatively simple index of aeration status. In our study, the potential for denitrification loss from three soils of widely varying texture were better indicated by WFPS rather than either gravimetric or volumetric water content [Fig. 3(b)].

Our data further illustrate that when denitrification losses were plotted against WFPS, substantial differences in denitrification losses in three soils of varying texture occurred only at 90% WFPS in vetch-amended soils [Fig. 3(b)]. These differences could be largely attributed to differential NO_3^- supply and varying response to added C between soils during the incubation. Cumulative C mineralized was 934 mg C kg^{-1} for the SiC in Houston Black SiC, 866 for the Gurdaspur SiL and 840 for the Tolewal SL with residue and 111, 137 and 72 mg C kg^{-1} without residue, respectively, an order identical to that observed for cumulative denitrification losses in residue-amended soils. In residue-amended soils, most soil NO_3^- was removed by denitrification and immobilization at 10 d, except in Houston Black SiC (Table 4). Differences in denitrification in vetch-amended soils at 90% WFPS [Fig. 3(b)] suggest the influence of soil texture on denitrification, at presumably equivalent states of soil aeration, was largely due to the complex interactions between the soils' inherent capacity to supply NO_3^-, available C, and other "soil factors".

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