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Influence of stock camping behaviour on the soil microbiological and biochemical properties of grazed pastoral soils

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Abstract The size and activity of the soil microbial biomass in grazed pastures was compared on the main grazing area and on stock camp areas where animals congregate. Two sites were on hill country and three on gently sloping border-dyke irrigated land. Due to the transfer of nutrients and organic matter to the camp areas via dung and urine there was an accumulation of soil organic C, organic and inorganic P and S and soluble salts in the camp areas. Soil pH also tended to be higher in camp areas due to transfer of alkalinity by the grazing animals. Water soluble organic C, microbial biomass C and basal respiration were all higher in soils from camp areas but the proportion of organic C present as microbial C and the microbial respiratory quotient were unaffected. Microbial activity as quantified by arginine ammonification rate and fluorescein diacetate (FDA) hydrolysis was higher in camp than non-camp soils but dehydrogenase activity remained unaffected. Activities of protease, histidase, urease, acid phosphatase and aryl-sulphatase were all higher in stock camp soils. The activities of both histidase and aryl-sulphatase were also higher when expressed per unit of microbial biomass C, indicating that the increased activity was the result of increased enzyme production by the microbial community. Prolonged regular applications of dairy shed effluent (diluted dung and urine from cattle) to a field had a similar effect to stock camping in increasing soil organic matter content, nutrient accumulation and soil biological activity. It was concluded that the stock camping activity of grazing animals results in an increase in both the fertility and bio-

logical activity in soils from camp areas at the expense of these properties on the main grazing areas.

Key words Grazing animals · Enzyme activity · Microbial biomass · Pasture · Soil organic matter

Introduction

Grazing animals have a dominant effect on the movement of nutrients through the soil / plant / animal system and thus the fertility of pasture soils (Haynes and Williams 1993). This is because animals only use a small proportion of the nutrients they ingest and 60–95% of ingested nutrients are returned to the pasture in the form of dung and urine. Dung and urine patches are therefore areas of pasture where nutrients are recycled from excreta to soil and back to pasture plants. In addition, large amounts of organic matter are deposited in dung patches and dung deposition plays a major role in the accumulation of soil organic matter that often occurs under improved pasture (Haynes and Williams 1993).

Since grazing animals cycle nutrients and organic matter back to pasture soils, one would expect their activities to promote soil biological activity. Experimental results have, however, been somewhat inconclusive. For example, Bristow and Jarvis (1991) found values for microbial biomass C (C_{mic}) were similar under cutting or grazing management whilst Kieft (1994) observed that grazing management had no consistent effect on either C_{mic} or basal respiratory rate. Similarly, in a field study using artificially placed dung pats, Lovell and Jarvis (1996) found no effect on C_{mic} or basal respiratory rate in underlying soil.

The pattern in which nutrients and organic matter are returned to the pasture as dung and urine is non-uniform. Animals generally deposit more excreta (and thus nutrients) on areas where they congregate (i.e. stock camps) such as beneath trees and hedges, around gateways and water troughs on areas away from road-

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sides and on ridges and hillcrests on hill country farms (Rowarth et al. 1992). This transfer of excreta to stock camps is usually the major mechanism for nutrient loss from grazed pastures. Indeed, because of the greater excretal returns there is typically an accumulation of available nutrients and organic matter in soils of camp-site areas (Haynes and Williams 1993). Thus, if the activities of grazing animals influence soil biological activity it is most likely to be evident in stock camp areas.

On intensive dairy farms under rotational grazing on flat land there is little opportunity for the cows to camp. However, dairy shed effluent is often sprayed over fields close to the shed. Since such effluent is a mixture of dung, urine and wash-water, spraying effluent over some fields would be expected to have a similar effect to the camping activities of animals.

The purpose of this study is to compare the size and activity of C_{mic} in stock camp and non-camp areas of developed pastures grazed with sheep. In addition, a dairy pasture soil receiving regular applications of dairy shed effluent is compared with one not receiving effluent.

Materials and methods

Soils were collected from six sites in the South Island of New Zealand (Fieldes 1968). Sites 1 and 2 were on a hill country sheep farm in the North Canterbury region. The soil at the site was a Hurunui silt loam (Typic Dystrachrept; USDA taxonomy). The two sites were on similar terrain but about 1 km apart. The main grazing area was a sloping field (about 40°) and the camp site was at the crest of the slope close to a water trough. Sites 3, 4 and 5 were on a Templeton silt loam (Udic Dystrachrept) grazed by sheep located in the Templeton Agricultural Research Station in mid-Canterbury. Sites 3 and 4 were about 1 km apart in border dyke-irrigated fields. Soils were sampled in the main grazing area and on the raised levee area where sheep camp. Site 5 was in a field adjoining a woolshed 400 m from site 3. At site 5, soils were sampled in the main grazing area and in an area close to the woolshed wall where grazing sheep are known to congregate. Site 6 was on a dairy farm in mid-Canterbury on a Coopers Creek silt loam (Mollic Haplaquent). A field close to the milking shed which had been regularly sprayed with dairy shed effluent for the past 10 years was sampled. A field approximately 200 m away that had never had effluent applied was sampled for comparison. All the study sites were under permanent pasture which consisted mainly of a mixture of perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.).

For sites 1–5, ten soil samples were randomly taken within the camp area and ten in an adjacent main grazing area. For site 6, ten samples were randomly taken in the effluent-treated field and ten from an adjacent untreated field. Soils were sampled to a depth of 20 cm and sectioned into the 0–5, 5–10, 10–15 and 15–20 cm layers. Within 48 h of collection, field-moist samples were sieved (<2 mm) and a sub-sample was air-dried and finely ground (<150 µm). Organic C, total P, and total S were measured on air-dried samples whilst all other analyses were performed on field-moist samples.

Organic C (C_{org}) was determined colorimetrically by the Walkley and Black method (Blakemore et al. 1972). Water-soluble C was extracted from soil with distilled water (1:5 soil:water ratio for 30 min), centrifuged, filtered through prewashed filter paper and measured by dichromate oxidation. Total soil P was measured following digestion of the soil with nitric and perchloric acids and total organic P was measured by the ignition method (Olsen and Sommers 1982). Phosphate in digests was determined

by the molybdenum-blue method. Phosphate-extractable sulphate was extracted with 0.03 M $Na_2H_2PO_4$ (1:5 soil:solution ratio for 16 h) and total S was measured on air-dried soil by dry ashing following procedures described by Tabatabai (1982). Soil pH was measured with a glass electrode using a 1:2.5 soil:water ratio and soluble salts were measured with a conductivity meter in a 1:5 soil:water ratio. C_{mic} was estimated by the method of Vance et al. (1987) based on the difference between C extracted with 0.5 M KCl from chloroform-fumigated and unfumigated soil samples using a K_c factor of 0.38. The microbial quotient (C_{mic}/C_{org}) was calculated by expressing C_{mic} as a percentage of total soil organic C. Basal respiration was determined by placing 30 g oven-dry equivalent of field-moist soil in 50-ml beakers and incubating the sample in the dark at 25 °C in a 1-l air-tight sealed jar along with 10 ml of 1 M NaOH. The CO_2 -C evolved was determined after 2, 5 and 10 days by titration with HCl. The microbial metabolic quotient (qCO_2) was calculated as basal respiration ($mg\ CO_2\ C\ h^{-1}\ g^{-1}\ C_{mic}$). The arginine ammonification rate was measured by the method described by Franzluebbers et al. (1995) using an incubation period of 3 h and a temperature of 25 °C. The assays of various enzyme activities were based on the release and quantitative determination of the product in a reaction mixture, the soil samples being incubated with suitable substrate and suitable buffer solution. Assays were performed to determine the activity of dehydrogenase, urea amidohydrolase (EC 3.5.1.5), acid phosphomonoesterase (EC 3.1.3.2) and arylsulphatase (EC 3.1.6.1) as described by Tabatabai (1994), casein hydrolysing protease (Ladd and Butler 1972) and L-histidine ammonia-lyase (EC 4.3.1.3) (Frankenberger and Johanson 1982). Enzyme activity was expressed as μmol product released g^{-1} soil h^{-1} . It was also calculated as $mg^{-1}\ C_{mic}\ h^{-1}$.

Sample means for the paired sites were compared using analysis of variance and the significance of differences was calculated using the Student's *t*-test at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$.

Results and discussion

Changes in C_{org} , soluble C, C_{mic} and histidase activity with depth on camp and non-camp areas of site 1 are shown in Fig 1. Values for these parameters were high-

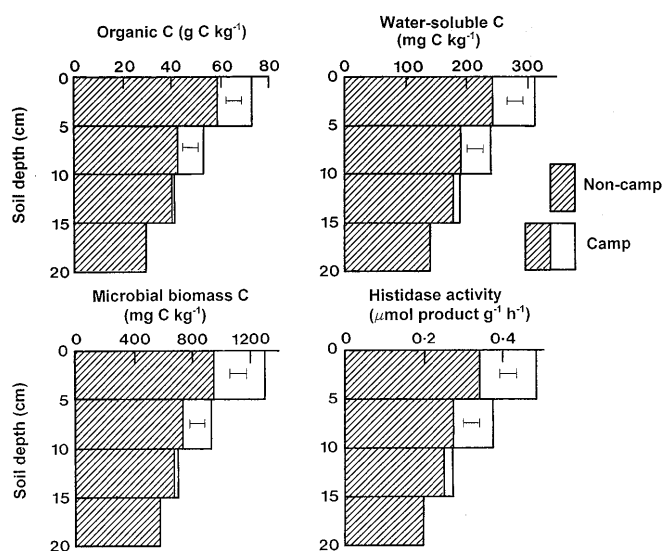


Fig. 1 Distribution of organic C, water soluble C, microbial biomass C and L-histidine ammonia-lyase activity in the soil profile on a hill-country pasture on the main grazed area (non-camp) and on a stock camp area. Magnitude of significant difference $P \leq 0.05$ shown based on the Student's *t*-test

Table 1 pH, soluble salt content and organic and inorganic P and S content of camp and non-camp areas and on an effluent-treated and untreated field under grazed pasture. Significance of camping effect shown: * $P \leq 0.05$, ** $P \leq 0.01$, *** $P < 0.001$

Site		pH (water)	Soluble salts (dS ⁻¹)	Organic P (mg P kg ⁻¹)	Inorganic P (mg P kg ⁻¹)	Organic S (mg P kg ⁻¹)	Adsorbed sulphate-S (mg S kg ⁻¹)
Hill country farm							
Site 1	Non-camp	5.4	0.50	611	844	431	6.5
	Camp	5.6 **	1.39 **	752 **	889 *	529 ***	10.8 **
Site 2	Non-camp	5.3	0.94	455	719	328	8.6
	Camp	5.6 ***	1.37 **	543 ***	793 **	386 *	16 **
Border dyke irrigated							
Site 3	Non-camp	5.8	0.79	614	626	358	6.8
	Camp	6.4 **	1.37 **	698 **	1010 ***	425 **	11 **
Site 4	Non-camp	5.8	0.54	614	565	345	5.7
	Camp	6.2 *	0.70 *	650 **	630 *	368 *	8.3 **
Woolshed field							
Site 5	Non-camp	6.1	0.70	633	832	427	5.4
	Camp	6.7 ***	1.10 *	741 *	986 *	489 **	7.6 **
Dairy effluent							
Site 6	Untreated	6.3	1.49	666	413	396	24
	Treated	6.4 ns	1.41 ns	751 *	489 ns	468 ***	26 ns

er in the camp area in the 0–5 and 5–10 cm layers but no significant differences were observed below 10 cm. This trend with soil depth is similar to that recorded at the other sites (i.e. sites 2,3,4 and 5) for these and the other measured parameters (data not presented). Since treatment differences were greatest in the 0–5 cm layer, results for this layer only are presented throughout the remainder of the paper. Soil C_{org} content decreased markedly down the soil profile and as a result so too did soluble C, C_{mic} , basal respiration, arginine ammonification, fluorescein diacetate (FDA) hydrolysis and the activity of all the enzymes assayed.

On hill-country farms, sheep graze the steep pasture slopes but camp on flat crests particularly around water troughs (Haynes and Williams 1993). Similarly, on border dyke – irrigated land, sheep graze the whole pasture area but camp on the raised levees (Williams and Haynes 1992) whilst on the field adjoining a woolshed, sheep had been observed to camp close to the woolshed wall. As shown here, the transfer of nutrients and organic matter (via animal excreta) from the main grazing area to the stock camps results in soils from stock camps having a higher available nutrient (i.e. inorganic P and S and soluble salts; Table 1) and organic matter status (C_{org} , P_{org} , S_{org} , water-soluble C; Table 2, Fig. 1). As expected, prolonged regular applications of dairy shed effluent (mainly diluted dung and urine from dairy cattle) to a field had a similar effect to the stock camping activity of sheep (Tables 1 and 2, Fig. 1). Accumulation of soil organic matter in stock camps is probably not only the result of transfers of or-

ganic matter in dung and to a much lesser extent urine, but also the higher soil nutrient status in the camp areas is likely to stimulate increased pasture growth and thus increased organic matter returns as above-and below-ground plant litter.

A slightly higher soil pH in stock camps compared with the main grazing areas was observed (Table 1). At first sight this is surprising since stock camps have a higher soil organic matter content and soluble salt content, both of which would tend to lower measured soil pH. The major factor contributing to the higher pH is transfer of alkalinity to stock camps by grazing animals (Sinclair 1995). In grass / clover pastures, N is supplied primarily by biological N_2 fixation by *Rhizobium* bacteria in the clover root nodules. Fertilizer N is seldom, if ever, applied. Because much of the herbage N is absorbed as N_2 rather than as negatively charged NO_3^- -N, the pasture absorbs a net cation excess from soil solution. In order to balance charge, H^+ ions are excreted into the rhizosphere (Haynes 1983). The plant herbage represents a quantity of alkalinity equivalent to the quantity of acidity imparted to the soil. This alkalinity is released in excreta soon after the pasture herbage is consumed by grazing animals. When excreta is deposited disproportionately in stock camps, a rise in soil pH occurs whilst the rest of the pasture soil is acidified (Sinclair 1995).

As expected the study soils, which were all under permanent pasture, had relatively high C_{org} levels (i.e. 35–75 g C kg⁻¹; Table 2) and C_{mic} / C_{org} ratios ranged from 1.6% to 2.9% (mean = 2.1). Each paired site in

Table 2 Organic C, water-soluble C, microbial biomass C and basal respiration in camp and non-camp areas and on an effluent-treated and untreated field under grazed pasture. Significance of camping effect shown: * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$

Site		Organic C (g C kg ⁻¹)	Water- soluble C (mg C kg ⁻¹)	Microbial biomass C (mg C kg ⁻¹)	Basal respiration ($\mu\text{g CO}_2\text{-C g}^{-1}\text{ day}^{-1}$)	Microbial quotient ($C_{\text{mic}}/C_{\text{org}}$) (%)	Microbial metabolic quotient ($\mu\text{g CO}_2\text{-C mg}^{-1}$ biomass day ⁻¹)
Hill country farm							
Site 1	Control	59	243	969	58	1.6	60
	Camp	73 ***	313 **	1271 ***	78 **	1.7 ns	61 ns
Site 2	Control	51	189	1123	60	2.2	53
	Camp	60 **	266 ***	1245 **	66 *	2.1 ns	53 ns
Border dyke irrigated							
Site 3	Control	35	276	1016	47	2.9	46
	Camp	41 *	308 **	1248 **	56 **	3.0 ns	45 ns
Site 4	Control	34	139	544	36	1.6	66
	Camp	37 *	168 *	687 **	43 *	1.8 ns	63 ns
Woolshed field							
Site 5	Control	37	139	791	36	2.1	46
	Camp	44 **	143 ns	860 **	44 *	2.0 ns	51 *
Dairy effluent							
Site 6	Control	63	211	1511	56	2.4	37
	Camp	75 **	303 ***	1799 ***	59 ns	2.4 ns	33 ns

this study appeared to have a characteristic $C_{\text{mic}} / C_{\text{org}}$ ratio which remained unaffected by stock camping (Table 2). This is because the increase in C_{mic} on stock camp areas was proportional to the increase in C_{org} . The reason for this may be that the study sites have been under long-term grazed pasture and have more-or-less come to an equilibrium soil organic matter level on both camp and non-camp areas. Similarly, $q\text{CO}_2$ was unaffected by camping activity (Table 2) but a characteristic value was attained for each paired site. It is notable that the two sites with the lowest $C_{\text{mic}} / C_{\text{org}}$ ratio (sites 1 and 4) had the highest $q\text{CO}_2$ values. Wardle and Ghani (1995) also observed that $q\text{CO}_2$ often increases as C_{mic} declines. That is, factors that are deleterious to the microflora tend to enhance its respiratory activity and vice versa. The different $C_{\text{mic}} / C_{\text{org}}$ ratios and $q\text{CO}_2$ values of adjacent sites (i.e. sites 1 and 2 or 3 and 4; Table 2) on the same soil type may be a result of differences in edaphic factors between sites. The $C_{\text{mic}} / C_{\text{org}}$ ratio can be affected by environmental factors such as soil pH, temperature and moisture content as well as soil clay and organic matter content, vegetation type, and soil management practice (Sparling et al. 1994).

Microbial activity was evaluated by measuring basal respiration, arginine ammonification, FDA hydrolysis and dehydrogenase activity (Tables 2, 3). Although dehydrogenase activity was unaffected by camping (Table 3), the other parameters were all higher in soils from stock camp areas. Dehydrogenase activity is often

correlated with microbial activity but in some cases no such correlation is observed (e.g. Falih and Wainwright 1996). One possible reason for the lack of correlations between microbial numbers and measured dehydrogenase activity may be due to the relative insensitivity of synthetic electron acceptors used in the assay.

The higher activities of enzymes involved in transformations of N, P and S on stock camp soils (i.e. protease, histidase, urease, acid phosphatase and arylsulphatase; Table 3) reflects a higher rate of turnover of these nutrients due to higher nutrient inputs in the form of dung and urine. Measurements on hill country farms have shown that about 60% of dung and 55% of urine is typically deposited on stock camp areas which occupy only 15–30% of the total land area (Haynes and Williams 1993). Nutrient balance studies on hill country pastures have shown that annual net nutrient transfers to camp sites can often amount to about 210 kg N ha⁻¹, 30 kg P ha⁻¹ and 15 kg S ha⁻¹ (Rowarth et al. 1992; Haynes and Williams 1993). The nutrient transfer to levees on border dyke-irrigated land is much less and was estimated by Williams and Haynes (1992) to be in the vicinity of 8.4 kg N ha⁻¹, 1.2 kg P ha⁻¹ and 0.6 kg S ha⁻¹ annually. Nonetheless, the levee area makes up a very small proportion of the total pasture (<10%) and consequently nutrient and organic matter concentrations in these small areas are high. Approximately 30%, 20% and 40%, respectively, of the N, P and S returned in excreta is in organic form. The higher activity of enzymes in-

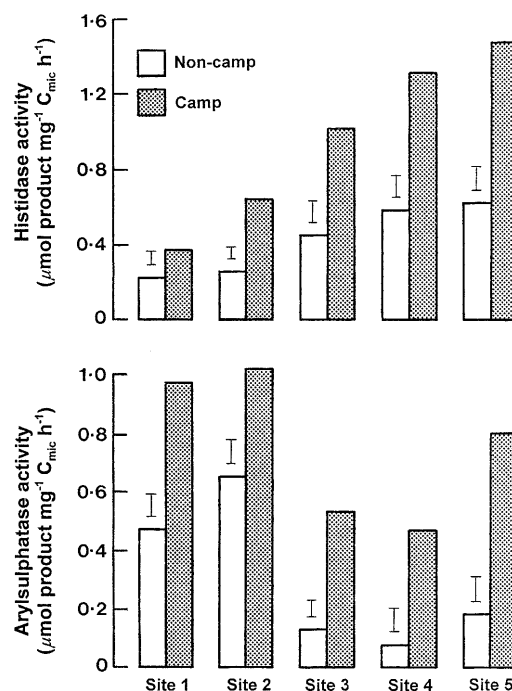
Table 3 Arginine ammonification rate, fluorescein diacetate (FDA) hydrolysis rate and the activity of dehydrogenase, protease, histidase, urease, acid phosphatase and arylsulphatase oncamp and non-camp areas and on an effluent-treated and untreated field under grazed pasture. Significance of camping effect shown: * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$

Site	Arginine ammonification rate	FDA hydrolysis rate	Dehydrogenase activity	Protease activity	Histidase activity	Urease activity	Acid phosphatase activity	Arylsulphatase activity
($\mu\text{mol product released g}^{-1} \text{h}^{-1}$)								
Hill country farm								
Site 1 Control	0.34	0.71	0.039	1.08	0.34	3.3	17	0.64
Camp	0.51 **	0.79 *	0.037 ns	1.42 **	0.89 ***	3.8 *	24 **	1.31 ***
Site 2 Control	0.49	0.66	0.070	0.94	0.21	3.5	18	0.54
Camp	0.86 ***	0.74 **	0.073 ns	1.32 **	0.39 ***	4.2 **	26 **	1.21 ***
Border dyke irrigated								
Site 3 Control	0.62	0.48	0.079	0.78	0.44	3.2	10	0.15
Camp	0.78 **	0.53 *	0.081 ns	1.04 **	1.27 ***	3.6 *	17 *	0.67 ***
Site 4 Control	0.56	0.57	0.065	0.62	0.39	2.6	8	0.05
Camp	0.69 ***	0.65 ***	0.068 ns	0.85 *	0.98 ***	3.3 **	14 **	0.34 ***
Woolshed field								
Site 5 Control	0.61	0.48	0.074	0.81	0.48	2.1	12	0.15
Camp	0.82 ***	0.60 **	0.079 ns	0.99 **	1.27 ***	3.9 ***	16 *	0.69 ***
Dairy effluent								
Site 6 Untreated	0.42	0.58	0.092	1.21	1.01	2.8	19	1.21
Treated	0.50 **	0.76 ***	0.095 ns	1.40 *	1.12 ns	3.3 **	28 **	1.53 **

involved in N, P and S mineralization will help cycle this input of nutrients. Nevertheless, the accumulation of organic N, P and S in stock camp soils suggests that net immobilization and/or direct accumulation of organic forms of these nutrients has been occurring.

Enzyme activity is often closely related to C_{org} and C_{mic} (Tabatabai 1994), so the increased activities in stock camp soils were not unexpected. For histidase, the enzyme activity expressed per unit of C_{mic} increased in camp soil (Fig. 2), demonstrating that the increase was not only the result of a larger C_{mic} but also a higher rate of enzyme production by the microbial community. Histidase catalyses the irreversible non-oxidative deamination of L-histidine to urocanate and NH_4^+ and is important as a last step of N mineralization (L-histidine comprises about 10% of total basic amino acids found in soil hydrolysates) (Frankenberger and Johanson 1982). By contrast, casein-hydrolysing proteases and urease activity increased in proportion to that for C_{mic} (data not shown). The enhanced urease activity in the camp areas may well enhance the opportunity for gaseous losses of NH_3 in urine patch areas. Urine is a concentrated N solution (about 10 g N l^{-1}) of which 80–90% is urea. Losses of 15–25% of urine N via NH_3 volatilization are common in urine patch areas of pasture (Haynes and Williams 1993).

The increase in acid phosphatase activity in camp areas is of note since there is also a much higher level of inorganic P (and extractable Olsen P; data not shown)

**Fig. 2** L-Histidine ammonia-lyase and arylsulphatase activity in soils (0–5 cm) from non-camp and stock camp areas of five pastoral sites expressed per unit (mg) of microbial biomass C. Magnitude of significant difference $P \leq 0.05$ shown based on the Student's *t*-test

in the soils in these areas. An increase in available soil P is often accompanied by a decrease in phosphatase activity since orthophosphate is a competitive inhibitor of acid phosphatase (Tabatabai 1994). It is therefore not surprising that the phosphatase activity to C_{mic} ratio was not increased (data not shown). By contrast, arylsulphatase activity is not greatly affected by high concentrations of soil SO_4^{2-} (Tabatabai 1994) and as a result the arylsulphatase activity when expressed per unit of C_{mic} was increased in soils from stock camps (Fig. 2).

It is concluded that the transfer of nutrients and organic matter from the main grazing area to stock camps by grazing sheep not only results in a diminution of nutrient status in the main grazing area but also in a decline in soil biological activity relative to that in the camp area. That is, both chemical and biological indices of soil quality are decreased on the main grazing area and increased on camp areas. Regular applications of dairy shed effluent to some fields on dairy farms has a similar effect to camping in increasing nutrient and organic matter inputs and enhancing soil biological activity.

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