

Vadose Zone Processes and Chemical Transport

Mobility of Soil Nitrogen and Microbial Responses following the Sudden Death of Established Turf

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ABSTRACT

The stability of nitrogen within a turf-soil ecosystem is important both for efficient turf management and preventing the contamination of ground water by nitrate. The objective of this study was to quantify responses of the microbial community and the mobility of soil nitrogen following the sudden death of established turf. Twelve-year-old turf plots comprising four cool-season turfgrass species fertilized with five N sources were maintained on an Enfield silt loam (coarse-silty over sandy or sandy-skeletal, mixed, active, mesic Typic Dystrudept) at Kingston, RI. Half of the plots were killed with glyphosate in early September and any regrowth was removed mechanically. Measurements of soil physical, chemical, and microbiological properties and nitrate leaching in killed and healthy plots were compared for 12 mo. Turf death did not alter soil moisture, temperature, pH, or extractable ammonium. Nitrate levels were higher in both the root zone and at 60 cm following turf death and this difference persisted for the sampling year. Carbon mineralization and microbial biomass C were not different between soils from healthy and killed plots. Killed plots leached three times more nitrate than healthy plots but this amounted to less than 10% of total soil N present. Retention of nitrate in a turf-soil system depends on absorption by living grass roots, although reasonable N stability is also provided by N cycling within the soil microbiota. Protecting ground water from nitrate contamination is optimized by maintaining a vigorous turfgrass cover.

RESEARCH on the fate of fertilizer nitrogen (N) applied to turf has been extensively reviewed by Petrovic (1990) and an analysis of soil water samples from 36 golf courses throughout the USA was reported by Cohen et al. (1999). Both conclude that soil water leaching from managed turf sites has a median nitrate N concentration of $<0.5 \text{ mg L}^{-1}$ and rarely exceeds the maximum contaminant limit (MCL) for potable water of $10 \text{ mg NO}_3\text{-N L}^{-1}$ established by the USEPA (Hallberg, 1989). Our research has shown that when using best management practices, nitrate leaching is equivalent to about 2.5% (4.4 kg N ha^{-1}) of fertilizer N applied annually (Hull et al., 1993). This is about twice the amount of nitrate lost to ground water from an unfertilized native hardwood forest in New England (Gold et al., 1990). Such findings place managed turf among the more environmentally sound land uses over shallow aquifers where potential for ground water contamination is great.

These conclusions are based mostly on short-term

studies in which turf was fertilized with specific nitrogen sources at defined rates for 2 or 3 yr. In a previous study, we monitored all known routes of nitrogen loss from turf during 5 yr. Gaseous losses, including both ammonia volatilization following fertilizer application and denitrification during and after heavy precipitation events, accounted for ~6% of N applied, with nitrate leaching accounting for an additional 5% (Hull et al., 1993). Surface runoff is rarely observed from a dense turf on a well-drained soil (Linde et al., 1998). Clipping removal from turf represents a major N loss, equivalent to as much as 60% of that applied as fertilizer (Petrovic, 1990). However, if clippings are retained on turf and fertilizer N is applied annually at 150 to 200 kg ha^{-1} over many years, N would be expected to accumulate to levels that would make further fertilizer applications unnecessary. This apparently does not occur, leading to the expectation that long-term fertilization of turf may result in greater N losses than can be detected in short-term studies. An alternative explanation may be that a turfgrass ecosystem is capable of substantial N accumulation in forms not available to turfgrasses when their need is greatest.

The latter theory is supported by our observation that total soil N accumulated to $>2240 \text{ kg ha}^{-1}$ in a long-established turf-soil ecosystem ($<30 \text{ yr}$) following 4 yr of intensive management involving annual applications of 170 kg ha^{-1} (Hull and Liu, 1995). Similarly, Porter et al. (1980) observed a progressive increase in soil N as turf stands aged, but after 25 yr they reached a plateau of about $2750 \text{ kg N ha}^{-1}$. Values in excess of $4000 \text{ kg N ha}^{-1}$ were recorded, however.

The stability of N within the turf-soil ecosystem in the event of sudden turf death could be a matter of concern. Prolonged drought coupled with severe insect predation of grass roots could result in widespread turf destruction and the potential for extensive N mineralization and nitrate leaching. Herbicide misapplication, prolonged flooding, disease epidemic, and renovation practices could have the same result. If significant amounts of N in fresh soil organic matter are mineralized and oxidized, with no living turfgrass roots present to absorb the resulting nitrate, leaching could be heavy and nitrate contamination of ground water severe. This was observed in a New Hampshire forest ecosystem following herbicide destruction of all vascular plants (Likens et al., 1970). To our knowledge, the impact of a sudden turf death scenario on the potential for nitrate leaching has never been reported. The research presented here

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investigated the effect of turf death on nitrogen stability within a turf-soil ecosystem. Because microorganisms may mediate the release of soil nitrogen through mineralization of organic matter, we also evaluated the effect of turf death on carbon mineralization rate (a measure of microbial activity) and microbial biomass. A worst-case scenario is presented in which no efforts are made to reestablish turf for 1 yr following its death.

MATERIALS AND METHODS

Experimental Plot Area

Field plots used for this study were seeded on 20 June 1985 and consisted of four turfgrass species: 'Georgetown' Kentucky bluegrass (*Poa praeensis* L.), 'Jamestown' Chewings red fescue (*Festuca rubra* var. *commutata* Gaud.), 'Repell' perennial ryegrass (*Lolium perenne* L.), and 'Scaldis' hard fescue (*Festuca longifolia* Thuill.). Between 1985 and 1990, these plots received minimal fertilization and were never irrigated. Prior to 1985, the plot area had been in Kentucky bluegrass fertility trials (two plantings) extending back to 1966.

The immediate predecessor to the present study was initiated in the fall of 1990, when five N fertilizers were applied at 120 kg N ha⁻¹ in early or late fall. The N sources included readily mineralizable (urea), slowly hydrolyzable (IBDU [Vigoro Industries, Fairview Heights, IL] and Coron [Helena Chemical, Memphis, TN]), and composted (Earthgro [Lebanon, CT] Lawn Food) or digested (Milorganite [Milorganite Division, MMSD, Milwaukee, WI]) sewage sludge. All N fertilizers were also applied at 50 kg N ha⁻¹ during mid-June (total = 170 kg N ha⁻¹ yr⁻¹). A set of unfertilized control plots was also included. Except for periodic sample removal, all clippings were retained on the plots. The objective of the study was to determine the effect of turfgrass species, N source, and time of application during the fall on nitrate leaching.

The soil at this location is an Enfield silt loam. The field plot design was a split-plot with six fertilizer treatments (five nitrogen sources and an unfertilized control) and two fall fertilizer applications (early September and mid-November) constituting 12 randomly positioned 1.8- × 9.6-m main plots. Each main plot was randomly subdivided into four 1.8- × 2.4-m subplots comprising the four turfgrass species. The experiment was replicated four times.

On 9 Sept. 1997, all early fall fertility plots were not fertilized but sprayed with the nonselective herbicide glyphosate [*N*-(phosphonomethyl)glycine] at 3 kg ha⁻¹. The late fall fertility plots (half of the study area) were not sprayed. No fertilizer applications were made to any plots after the date of spraying.

Soil Water Sampling

Two replications (96 plots) were instrumented with suction lysimeters inserted in the soil to a depth of 60 cm. The lysimeters cups were below the root zone of all turf species and sampled water that had passed through the turf and was free to leach into the aquifer. Lysimeters consisted of a porous ceramic cup (2.24-cm o.d., 6.98-cm long) mounted on a PVC pipe (2.0-cm o.d., 53-cm long). These were inserted vertically in the soil so the open end, closed with a rubber stopper, was at the level of the soil surface. Twice each month from September 1997 through May 1998 (weather permitting), soil water samples were collected from the evacuated lysimeters and analyzed for nitrate using a cadmium-copper reduction column. The resulting nitrite was assayed spectrophotometrically (Keeney and Nelson, 1982). Because ammonium was of less concern for leaching and was present at much lower

concentrations than nitrate, it was not analyzed in the 60-cm soil water samples.

Nitrate leaching was estimated for each of the 96 plots by multiplying the volume of water percolating through the soil by the nitrate concentration of soil water sampled after the most recent leaching event. Leachate volumes were based on soil and meteorological data using a hydrologic mass balance model (GLEAMS; Leonard, et al., 1987). Soil parameters required by the GLEAMS model have been derived for our soil type from previous studies (Gold et al., 1990). By subtracting daily evapotranspiration from precipitation and irrigation, any water in excess of that required to bring the soil to field capacity was identified as leachate.

Soil Sampling

Out of 192 subplots, 72 randomly selected plots (36 killed and 36 healthy) were sampled on eight dates throughout the 12-mo period following turf death (Fig. 1) for analysis of soil properties and processes. A 2.5-cm diameter, stainless steel coring device was used to take soil samples to a 10-cm depth. Sampling was conducted between 0900 and 1200 h EST. Surface (top 10 cm) soil temperature was recorded using an analog soil thermometer. Soil samples were placed in sealable plastic bags and taken directly to the laboratory after collection. All visible plant material was removed and the soil mixed thoroughly to ensure physical homogeneity. Carbon mineralization, microbial biomass C, and soil moisture analyses were conducted on the day of sample collection. Soil used for all other analyses (NO₃⁻-N, NH₄⁺-N, and pH) was stored in the dark at 4°C for no more than 3 d before analysis.

Inorganic Nitrogen

The soil ammonium and nitrate content was determined following the method of Keeney and Nelson (1982). Fresh soil (1 g) was extracted with 10 mL of a 2 M KCl solution for 30 min. The resulting mixture was passed through a Whatman #42 filter. The filtrate was analyzed for ammonium and nitrate colorimetrically using an automated analyzer (Alpkem [Clackamas, OR] Model RFA 300).

Carbon Mineralization

Fresh soil (5 g) was placed in a 20-mL glass headspace vial and the vial was stoppered with a rubber septum and sealed with an aluminum crimp collar. The sealed vials were incubated at field temperature in a dark environmental chamber for 10 d. Görres et al. (1998) showed that incubation of soil samples under these conditions does not result in a significant decline of oxygen level in the vial headspace. Following incubation, the CO₂ concentration in the headspace of the vial was measured. A 1.0-mL sample of gas from the headspace of the vial was removed by displacement using an automated headspace sampler (Tekmar [Cincinnati, OH] Model 7000). The concentration of CO₂ in the headspace gas sample was determined with a gas chromatograph (Shimadzu [Kyoto, Japan] Model 14 A) fitted with a Porapak Q column (80/100 mesh, 3 m) (Alltech Assoc., Deerfield, IL). Carbon dioxide was converted to methane using a heated (400°C) Ni catalyst and an H₂ gas stream, and the resulting methane was measured with a flame ionization detector. Injector, column, and detector temperatures were 150, 60, and 300°C, respectively. Peak areas for CO₂ were determined by electronic integration. Conversion of peak area to mass of CO₂ was made by comparison with vials containing a known CO₂ concentration.

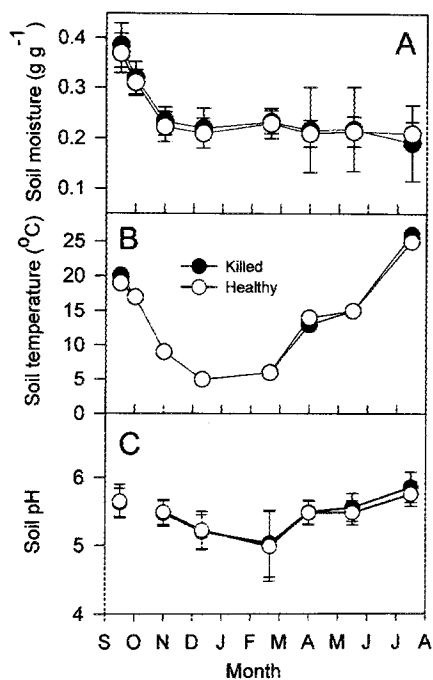


Fig. 1. Gravimetric moisture content (A), temperature (B), and pH (C) in surface soil of killed and healthy turf plots. Values shown for pH and moisture content are means ($n = 36$). Bars represent one standard deviation.

Microbial Biomass Carbon

The fumigation-incubation method modified from Jenkinson and Powlson (1976) was used to determine microbial biomass C. After the initial 10-d incubation used to determine C mineralization rate, the vials were uncapped and fumigated overnight with chloroform, evacuated to remove chloroform residues (replacing it with fresh air five consecutive times to ensure chloroform removal), and resealed and incubated for another 10 d. The CO_2 concentration was again measured following the second incubation and the CO_2 produced was subtracted from that produced during the initial incubation period. The difference was used to calculate microbial biomass C using a k_{EC} value of 0.40 (Sparling and West, 1988).

Soil Moisture and pH

Soil moisture was determined gravimetrically by drying 5 g fresh soil at 105°C overnight. To determine soil pH, 1 g fresh soil was mixed with 5 mL deionized distilled water. The mixture was allowed to equilibrate for 1 h and the pH of the supernatant solution determined with a pH meter.

Statistical Analyses

All data collected from this study were subjected to an analysis of variance for a split-plot design with significantly different means separated by the Waller-Duncan k -ratio t test using SAS (SAS Institute, 1990). Because no differences between the original experimental variables (fertilizer treatments and grass species) showed any consistently significant differences, all differences between healthy and killed turf shown here were evaluated for significance using a simple t -test (SAS Institute, 1990).

RESULTS

Turf sprayed with glyphosate on 9 Sept. 1997 was brown and presumably dead within 20 d. Throughout

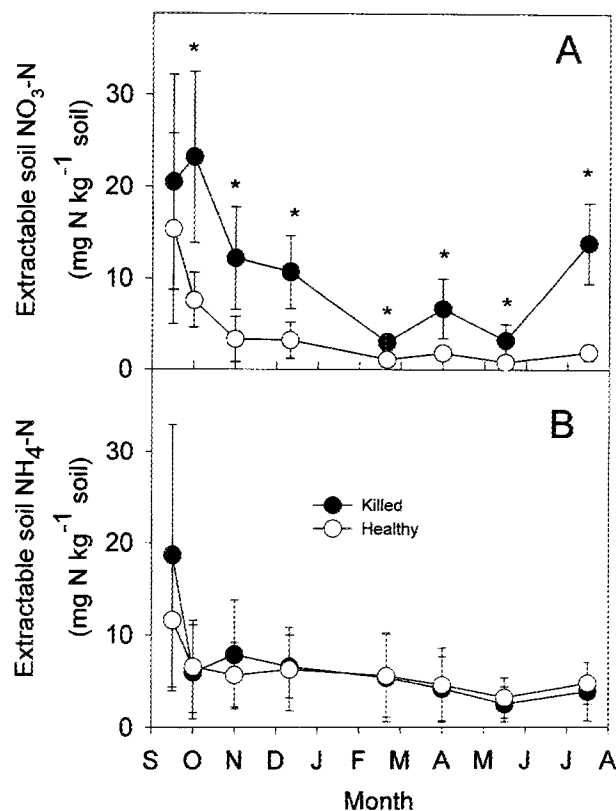


Fig. 2. Levels of nitrate (A) and ammonium (B) in surface soil of killed and healthy turf plots. Values shown are means ($n = 36$). Bars represent one standard deviation. Significant differences are indicated by an (*).

the 12-mo period following turf death, soil properties within the root zone (upper 10 cm) were recorded. No significant differences in soil temperature, soil moisture or soil pH were observed between killed and healthy plots on any sampling date (Fig. 1).

During the late fall and winter, earthworm activity was extensive, virtually covering the dead turf of perennial ryegrass plots with castings such that by late February the plots appeared to be 80% bare ground. Kentucky bluegrass plots also experienced earthworm activity but only about 50% of the surface was covered with castings. Dead turf on fine-leaved fescue plots was only 25% covered with castings, suggesting less earthworm activity or a dead grass stubble of more resistant structure.

Soil nitrate levels within the root zone (upper 10 cm) were higher in killed than in healthy plots on all sampling dates subsequent to the start of the experiment (Fig. 2a), with nitrate dynamics differing among treatments. Levels of nitrate in healthy plots decreased rapidly during the autumn and remained low and relatively constant during the winter, spring, and summer. By contrast, soil nitrate levels in killed plots declined at a relatively constant slower rate during the autumn and winter, increasing again in the spring and summer. We did not observe differences in levels of soil ammonium between healthy and killed plots on any sampling date (Fig. 2b). The soil ammonium content declined steadily during the autumn, winter, and spring, increasing again slightly in the summer. Nitrate constituted more than

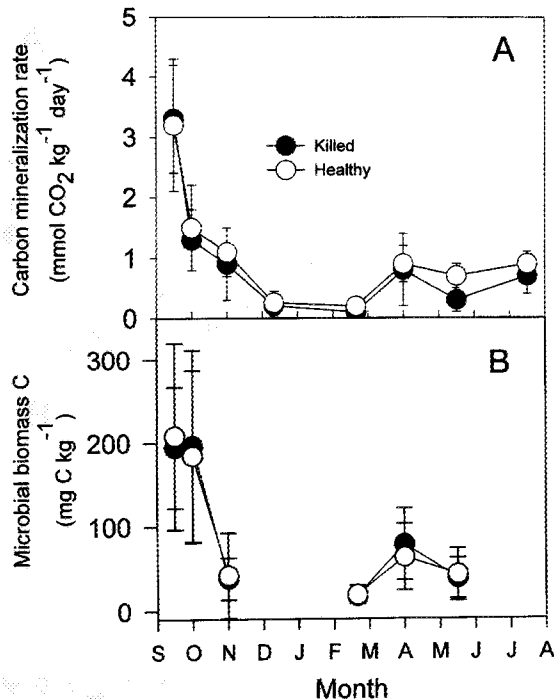


Fig. 3. Carbon mineralization rate (A) and microbial biomass C (B) in surface soil of killed and healthy turf plots. Values shown are means ($n = 36$). Bars represent one standard deviation.

50% of the measured inorganic N ($\text{NH}_4^+ + \text{NO}_3^-$) under killed turf from September to December and again in July (Fig. 2). In healthy plots, inorganic N was more than 50% nitrate only in September and October.

Carbon mineralization rates were not different between healthy and killed plots on any sampling date (Fig. 3a). The temporal pattern of C mineralization for both treatments generally followed that for soil temperature, with rates declining as soil temperature declined from September through February and increasing during the spring and summer. The increase in C mineralization subsequent to the winter season was not as high as expected based on soil temperature (Fig. 1b), probably as a result of limiting soil moisture (Fig. 1a). No differences in microbial biomass C between healthy and killed plots were observed (Fig. 3b). Dynamics of microbial biomass C followed those of C mineralization.

The nitrate concentrations of soil water recovered below the root zone (60 cm) of healthy and killed turf did not differ until 3 Nov. 1997, almost 2 mo following herbicide application (Fig. 4). From then through 21 Sept. 1998, the killed plots consistently yielded water higher in nitrate N than did the healthy plots. During the late fall and early winter, nitrate leaching from killed plots averaged three to four times the MCL established for nitrate N while that from healthy plots never exceeded the MCL of $10 \text{ mg NO}_3\text{-N L}^{-1}$. Even unfertilized killed plots leached nitrate two times in excess of the MCL during early winter (data not shown). While nitrate levels of soil water leaching through the root zone decreased during the late winter and early spring in both killed and healthy plots, they both increased again during the late spring and summer, with killed plots maintaining higher nitrate levels.

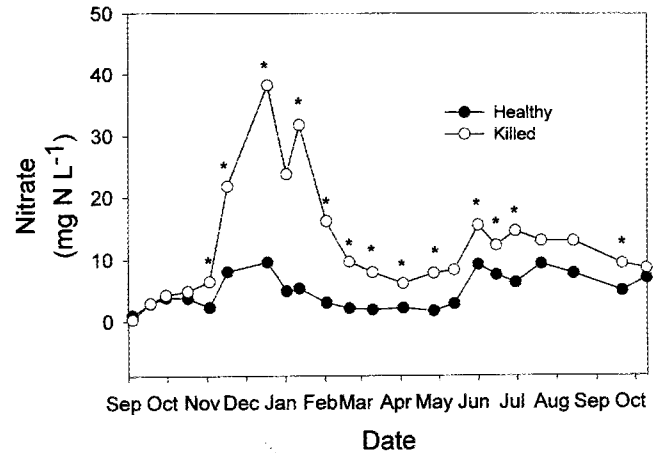


Fig. 4. Nitrate N concentrations of soil water sampled via suction lysimeters at a 60-cm depth from killed and healthy turf plots. Values shown are means ($n \leq 48$). Significant differences are indicated by an (*).

The species of grass, healthy or killed, had no effect on the nitrate concentration of water percolating through the turf. The N source applied during the preceding 7 yr exerted minimal effects on the nitrate concentration of soil water recovered below the root zone (data not shown).

Estimates of nitrate leaching during the year following turf death indicated that killed turf leached almost $161 \text{ kg nitrate N ha}^{-1}$ compared with 50 kg ha^{-1} from healthy plots (Fig. 5). Nitrate leaching from killed plots exceeded that from healthy plots throughout the sampling period, but the differences were greatest during the late fall and winter following turf death. Potential for heavy leaching from dead turf persisted into the following summer as evidenced by the nitrate losses recorded during the excessively wet June of 1998.

DISCUSSION

The absence of a treatment effect on C mineralization rates or microbial biomass C following turf death was surprising. Glyphosate has minimal effects on soil microflora over short-term (32 d; Rueppel et al., 1977) and long-term (214 d; Roslycky, 1982) exposures, except at extremely high concentrations (1000 mg kg^{-1} soil). Thus, it is unlikely that the herbicide application masked the effects of turf death on soil microflora by stimulating microbial activity or growth in killed plots. It is generally assumed that a fraction of the C used for energy and biomass increase by microorganisms comes from the turnover of roots and from root exudates (Tate, 1995). The flow of C from plants to the microbial community may occur partially from aboveground deposition of plant detritus in the turfgrass ecosystem (e.g., the thatch layer). Alternatively, the labile fraction of soil organic matter may be the main source of C. Our data generally support a hypothesis involving either thatch or soil organic matter as C sources.

Although killing the grass eliminates the flux of carbon through translocation of photosynthate to roots, it should have no short-term effect on the standing pools

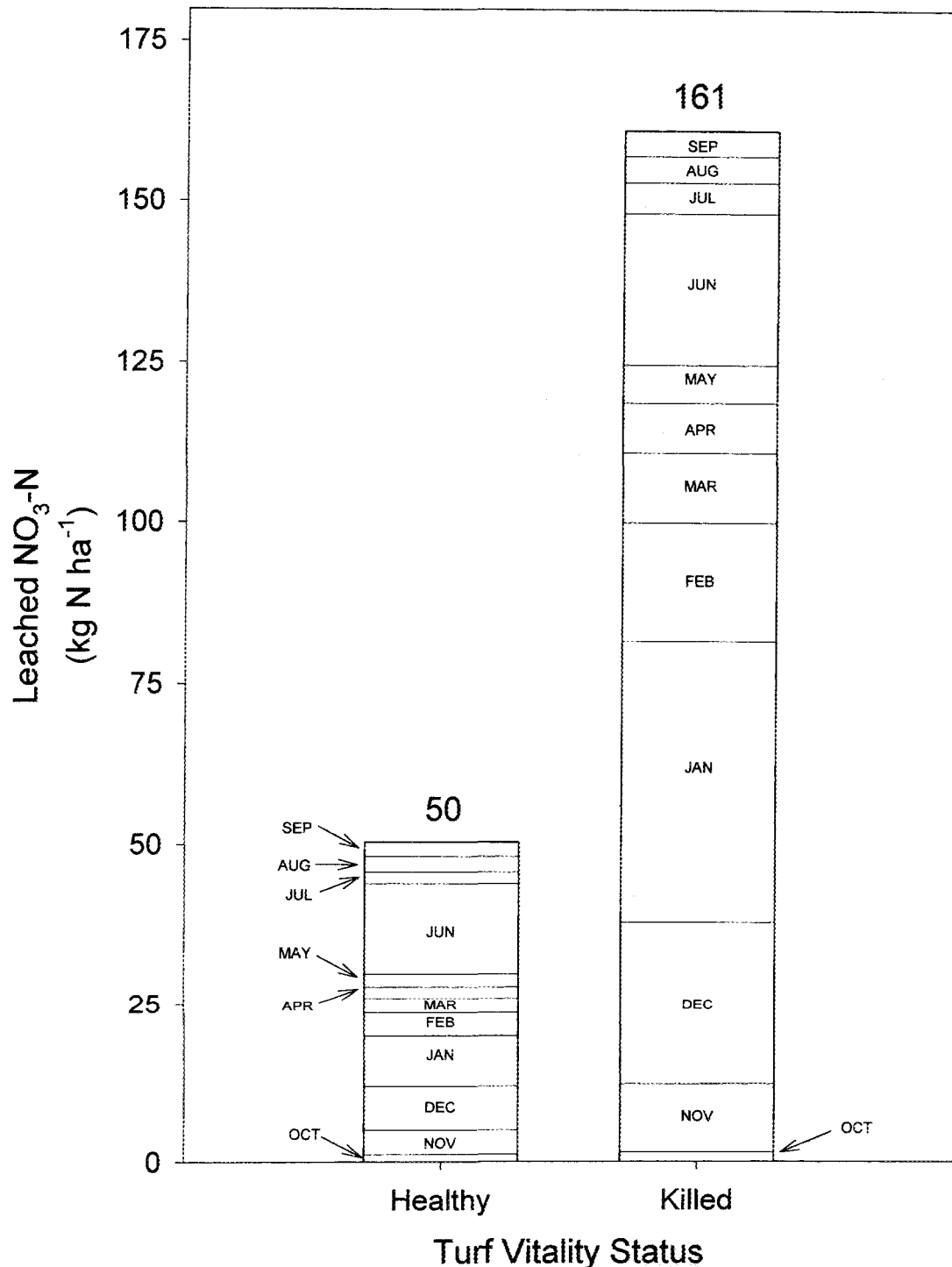


Fig. 5. Nitrate N leached from healthy and killed turf over a 12-mo period. Each horizontal bar is the cumulative N leached during the month indicated.

of C in the soil organic matter or thatch layer. If these two pools are the main sources of C, mineralization of C and synthesis of microbial C should not be affected by killing the grass, as we observed. Earlier studies (Hull and Smith, 1974; Hull, 1996) have indicated that photosynthate translocation in cool-season turfgrasses drops off sharply during the hot conditions of mid to late summer and this results in a normal loss of more than

half of the living root mass (Falk, 1976). June and July of 1997 were hotter and drier than normal and while August was wet and cool, turf roots probably had already seriously declined. When the grass was killed in September, the amount of root biomass contributed to the labile soil organic matter pool was probably small. Thus, the failure to perturb soil metabolism by killing the turf cover in late summer may not be so surprising.

An identical increase in microbial biomass C and C mineralization rates in the spring for both healthy and killed plots indicates that the additional plant detritus in killed plots and/or the photosynthate produced in the healthy plots either contribute comparable resources to soil microbes or do not contribute significantly to soil microbial activity. The contribution of photosynthate C to soil respiration varies radically among plant species and seasonally (Tate, 1995).

The absence of a treatment effect on ammonium levels suggests that the balance of processes producing ammonium (e.g., microbial ammonification and excretion by soil microfauna) and those consuming it (e.g., plant uptake, microbial immobilization, nitrification) was not altered by turf death. The failure of turf death to affect C mineralization, which is generally believed to be coupled to N mineralization (Gale and Gilmour, 1988), indicates that N mineralization to NH_3 was not affected by plant death. The absence of plant uptake would have been expected to result in increased accumulation of ammonium in the soil. It did not. Thus, it may be that nitrification was able to keep pace with any increased ammonium production, which would explain why nitrate accumulated to a greater extent in the soil of killed plots than in healthy plots.

Higher nitrate levels in killed plots persisted for the duration of the experiment. There were no differences in soil temperature, moisture level, or pH between healthy and killed plots, allowing us to dismiss these variables as explanations for the differences in nitrate level between living and killed plots. The level of nitrate in soil is a function of production (nitrification) and consumption (microbial assimilation, plant uptake, denitrification). The most obvious explanation for these results is the absence of nitrate uptake by plants, which represents a sizable sink for this form of nitrogen. Alternative explanations are not supported by our data. For example, decreases in microbial assimilation of nitrate are unlikely, given that there was no significant treatment effect on either microbial biomass C or C metabolism. Enhanced nitrification rates in the killed treatment would result in depletion of the ammonium pool, but we did not find differences in ammonium levels among treatments on any sampling date. Thus, it appears that nitrate accumulation resulted from lack of plant uptake.

The effects of turf death on increasing nitrate levels within the root zone translated into elevated nitrate in soil water leachate within 2 mo of applying glyphosate to the turf. This delay in greater nitrate leaching below the root zone was partly due to 58% less than normal precipitation during September and October. The increase in root zone soil nitrate within 2 wk of turf death (Fig. 2a) suggests that enhanced nitrate leaching would have been observed within that time if greater percolation had occurred. Water transport through the soil column is the environmental factor most responsible for nitrate leaching from turf (Morton et al., 1988).

The decline in soil water nitrate during the winter and early spring (Fig. 2a and 4) was probably due to reduced N mineralization caused by low temperatures and continuous nitrate leaching. We estimated that 630

mm of water percolated through the soil profile between January and May 1998 due to heavier than normal precipitation and few periods of frozen ground. Again, the consistently lower nitrate concentrations in deep soil water samples from healthy turf were due to nitrate absorption by roots. Substantial root activity in cool-season turfgrasses has been observed during the winter (Hull and Smith, 1974).

During the 12 mo following turf death, killed plots leached 2.7 times more nitrate than did healthy plots; 161 and 50 kg $\text{NO}_3\text{-N ha}^{-1}$, respectively (Fig. 5). The leaching from healthy turf was greater than that normally recorded for these or similar plots (Hull et al., 1993), where 20 kg N $\text{ha}^{-1} \text{yr}^{-1}$ was rarely exceeded. This might be due to the heavy winter precipitation and injury sustained by some healthy plots due to herbicide drift. While seriously damaged healthy plots were excluded from our analysis, some undetected damage may have occurred. All plots identified as healthy resumed normal vigorous growth in the spring, so any herbicide injury was at most transitory, although earlier it may have caused some inhibition of nitrate uptake.

The plots used in this study contained about 2250 kg N ha^{-1} in the soil organic fractions (Hull and Liu, 1995). Given this quantity of N within the turf–soil ecosystem, it is surprising that no more than 170 kg N were lost to leaching during this 12-mo period of fallow soil. This further supports the idea that soil microbial populations and activity rely heavily on soil organic matter and only to a minor extent on current inputs from roots or surface residues. The burying of dead perennial ryegrass sod under earthworm castings did not stimulate soil metabolism nor did it increase nitrate leaching since no species effects were observed. Apparently, microbial immobilization accounts for most mineralized N and only a relatively small amount is subject to nitrification and thereby rendered vulnerable to leaching or denitrification losses.

CONCLUSIONS

This research emphasizes the importance of nitrate absorption by turfgrass roots in stabilizing N within a turf–soil ecosystem. While turf death has little effect on soil microbial biomass or C, it eliminates a primary sink for mineralized N, resulting in elevated soil nitrate levels. If water percolation occurs, increased nitrate leaching will result. Even then, under a worst-case scenario, the amount of N lost to leaching is less than 10% of total N in the turf–soil ecosystem. While turf would normally be reestablished in less than a year following its death, these results indicate that the soil ecosystem retains N for some time in the absence of living plant roots. How quickly reseeded grass can stabilize soil N to the level of uninjured turf will be addressed in a following report (see Bushoven et al., 2000).

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