Urease Activity in a Kentucky Bluegrass Turf[1]

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ABSTRACT

The components of a turfgrass ecosystem, including plants, an intervening layer of thatch and the underlying soil, influence the fate of topically applied urea fertilizer. The loss of urea N by ammonia volatilization may be governed by the rate of urea hydrolysis. The main objective of this study was to determine the extent of urease activity associated with turfgrass plant tissue, thatch, and the underlying soil. This information may help elucidate the mechanism of ammonia loss following urea application. Because a turfgrass stand frequently possesses an extensive thatch layer that may serve as the primary plant growth medium, additional objectives included: i) determining the effects of air drying and seasonal variation on the activity of urease in thatch; ii) determining the variability in thatch urease activity by analyzing multiple field samples; and iii) determining the variation of urease activity within a thatch profile. Turfgrass clippings, thatch, and underlying Flanagan silt loam soil (Aquic Argiudoll) samples were taken from a field-grown Kentucky bluegrass (*Poa pratensis* L.) turf in either September 1980 or March 1981. On a dry weight basis, urease activity was 18 to 30 times higher from turfgrass clippings and thatch than from soil. Air drying thatch increased urease activity by 20% over moist samples while air drying soil samples had no apparent effect. Greenhouse incubation of winter-dormant thatch samples increased urease activity 40%, presumably in response to the duration of increased temperature. Thatch urease activity varied between sampling sites but still remained extremely high compared to soil activity. Within each thatch sample (1 × 1 × 2 cm), urease activity was highest in the upper 1.0 cm of the profile. It was concluded that thatch urease activity was variable in nature depending upon seasonal conditions which contrasts sharply with extremely stable soil urease activities. These findings suggest that, because of the high level of urease in thatch, ammonia volatilization will occur from most urea-treated turfgrass stands, regardless of the type of underlying soil unless the urea is thoroughly washed into the soil.

Additional index words: **Ammonia volatilization, Thatch, Nitrogen fate, *Poa pratensis* L.**

THE extensive use of urea as a nitrogen fertilizer in many agricultural endeavors has prompted numerous studies on the nature and activity of soil urease (urea amidohydrolase, EC 3.5.1.5). Bremner and Mulvaney (3) provided a comprehensive review of the characteristics of this enzyme. In general, soil urease activity can be affected by pH, temperature, and urea concentration (4, 14, 16). Soil urease activity has also been found to be extremely stable between a wide range of temperatures under 70°C and to increase dramatically with increasing levels of soil organic matter (9, 15).

Thatch has been defined as a tightly intermingled layer of living and dead stems, leaves, and roots of turfgrass which develops between the green vegetation and soil surface (2). This layer of organic matter in a turf develops as a result of a combination of various climatic, biological, and cultural factors (2).

Turgeon et al. (14) demonstrated that where a thick layer of thatch developed, it constituted the primary growing medium for turfgrass plants and the underlying soil was of secondary importance. Since a thatch layer can be an important growing medium, the characterization of urease activity in it could provide information on the availability of N after urea application. Information on urease activity within a thatch layer has not been previously reported.

Zantua et al. (18) suggested that most of the variation in urease activity among soils can be accounted for by the quantity of soil organic matter. Burns et al. (5), and Zantua and Bremner (17) implied that soil organic matter "protects" soil urease from microbial degradation without disturbing the enzyme's activity. Pettit et al. (9) postulated that soil urease is concealed within organic matter during humus formation and that the organic matter has pores large enough to facilitate diffusion of substrate and products, but not proteolytic enzymes.

The possibility of high levels of urease existing in thatch is excellent since thatch is an extensive layer of organic matter residing on the soil surface. High levels of urease activity in thatch may have a profound effect upon the fate of applied urea. An example is the fact that urea solutions that were sprayed on turf resulted in greater N loss due to ammonia volatilization than when prilled urea was applied at the same rate (13). It was hypothesized that this loss occurred through a mechanism involving high levels of urease activity originating from thatch or aboveground portions of turfgrass plants.

The primary objectives of this study were to determine the extent of urease activity associated with thatch and turfgrass plants and to compare these levels to those found in underlying soil. Secondary objectives were to characterize thatch urease activity by determining the effects of season and air drying of the thatch sample and to determine the variability of urease activity within a thatch profile and between field sampling sites.

METHODS AND MATERIALS

Soil and Thatch Descriptions

A 10-year-old stand of 'Kenblue' Kentucky bluegrass (*Poa pratensis* L.) was used in this study. Soil underlying the turfgrass stand was a Flanagan silt loam (Aquic Argiudoll) having a pH of 6.6 and a soil organic matter content of 6.0%. Pieces of sod (30 × 30 cm) with a 2.5-cm thick thatch layer were cut to a depth of 5 cm below the surface of the thatch during the first week of September 1980.

Pretreatment Procedures

Sod samples were placed in three brass containers measuring 30.8 × 31.9 × 5.0 cm (L × W × D). The containers were then placed within a growth chamber and maintained at a constant temperature of 20 ± 2.0°C (0.204 W m^{-2} light irradiance) and a 12-h light/12-h dark diurnal cycle. After an equilibration period of 48 h, each sod sample was treated with urea at a 49 kg-N ha^{-1} application rate (453.6 mg-N) and irrigated with 600 ml of distilled water. Samples were then allowed to incubate for 8 days prior to analysis of urease activity. Subsamples from urea-treated sod were air dried for 2 weeks prior to analysis to determine if drying and rewetting affected thatch urease activity.

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Soil underlying the urea-treated thatch was also analyzed for urease activity following the same pretreating and/or air drying sequences.

The results obtained from urea-treated thatch made it necessary to determine if similar urease activities were prevalent in untreated thatch. Sod samples were taken from the same location 1 week later than the first sampling and treated as described above but withholding urea application. Turfgrass clippings cut from the untreated sod samples were also analyzed for urease activity.

Characteristics of Thatch Urease Activity

The effect of seasonal variation on thatch urease activity was determined by comparing data taken in September 1980 to data obtained from winter-dormant turf samples analyzed directly from the field in March 1981. In that month, other thatch samples were taken into the greenhouse for either a 3- or 10-day period to observe the effects of increased temperature exposure on urease activity. Field temperatures during the March 1981 analysis did not exceed 10°C while greenhouse temperatures ranged between 20 and 30°C.

The variability of urease activity within a thatch profile was determined by sectioning thatch samples measuring 1 × 1 × 2 cm (L × W × H) into upper and lower 1-cm layers. Variability between sampling sites was determined by comparing thatch urease activity data from experimental plots 1 m apart. Samples for both these tests were extracted and analyzed for urease activity during March 1981.

Determination of Urease Activity

After pretreatment, thatch samples were prepared for analysis by first cutting away the turfgrass plants with a scissors and then cutting the thatch into 1-cm^3 block samples. Subsamples of thatch, soil, and turfgrass tissue were oven dried to determine moisture content. Thatch, soil, and turfgrass tissue samples were placed into separate 250-ml French bottles. To each bottle, 10 ml of a phosphate buffer (3.39 g KH_2PO_4 and 3.53 g Na_2HPO_4 L^{-1}) were added to hold the pH at 7.0. Urea substrate was added as 10 ml of a 1.6×10^{-2} M urea solution. Controls were treated in a similar manner except that 20 ml of a 250 ppm Ag_2SO_4 solution were added prior to substrate addition to inhibit urease activity. Incubation bottles were then stoppered and placed in a shaker-type water bath held at 32 ± 1.0°C and incubated for 5 h. Upon completion of each incubation period, 20 mL of a 250 ppm Ag_2SO_4 solution and 10 ml of a 2.0 M KCL solution were added to sample bottles. Since controls already had 20 ml of Ag_2SO_4 solution, only the KCl solution was added. The incubation bottles were then stoppered and agitated for 1 h on a mechanical shaker at 32 ± 1.0°C. After agitation, samples were centrifuged at 3500 rpm for 15 min after which 1 ml of supernatant from each sample was transferred into separate 25-ml volumetric flasks. One milliliter of each indicator solution (see below) was then added to each sample and flasks were brought to volume with deionized water.

The analysis of urease activity was based upon a colorimetric assay for ammonium described by Chaney and Marbach (6). The color developing reagents and concentrations are as follows: phenol (50 g L^{-1}), and sodium nitroprusside (0.25 g L^{-1}) constituted developing solution No. 1, while sodium hydroxide (25 g L^{-1}) and sodium hypochlorite (2.1 g L^{-1}) constituted developing solution No. 2. For color development, 1 mL of each developing solution was added to the samples. Color development was complete in approximately 3 min when heated in a water bath at 60°C. Spectrophotometric analysis was accomplished using a Beckman model 26 scanning spectrophotometer at an absorbance maxima of 625 nm. A standard curve was developed for NH_4^+ concentrations ranging from 0.02 to 0.64 ppm. Data obtained by this method were expressed as μg urea-N hydrolyzed g^{-1} sample dry wt $h.^{-1}$

Table 1. Urease activities associated with thatch, soil, and turfgrass clippings.

Treatment	% Dry wt.	Mean ($\bar{x}$) urease activity
		μg urea-N hydrolyzed g^{-1} sample dry wt h^{-1}
Soil	84.0	60.0 E*
Soil (air dried)	98.5	57.3 E
Thatch (untreated)	36.0	1797.5 B
Thatch (urea treated)	53.0	1486.5 C
Thatch (air dried, urea treated)	95.0	2179.5 A
Turfgrass clippings	24.4	1076.3 D

* Means ($\bar{x}$) followed by the same letter are not significantly different by Duncan's Multiple Range Test (P = 0.05).

Each treatment included at least three controls and 5 to 10 replications. Data were statistically analyzed using a completely randomized design. Mean ($\bar{x}$) values for each treatment were subsequently compared using Duncan's Multiple Range Test (7) or an LSD at the 0.05 level of confidence.

RESULTS AND DISCUSSION

On a dry weight basis, urease activities of thatch and turfgrass clippings were found to be extremely high and were, on average, 18 to 25 times greater than underlying soil urease activity (Table 1). Comparisons of urease activity on a dry weight basis were considered to be more precise than comparisons based on bulk densities. Hurto et al. (8) found the bulk density of thatch to be three to four times less than the underlying soil. If the urease activity of thatch was compared to that of soil on a bulk density basis [assuming a one-third thatch/soil bulk density ratio (Hurto et al. (8)], the results would still show thatch to have six to eight times more urease activity than underlying soil. The elevated levels of thatch urease activity may reflect the positive effects of organic matter on native urease by a protection mechanism described in detail by other researchers (5, 9, 17).

Comparisons of urease activities from urea-treated thatch, untreated thatch, and air dried thatch are included in Table 1. Untreated thatch was analyzed for urease activity 2 weeks after analysis of urea-treated thatch. The analysis of untreated thatch was necessary to determine "native" thatch urease activity levels. Untreated thatch samples had slightly higher levels of urease activity compared to urea-treated thatch. Prior to the analysis, however, untreated thatch samples were 17% wetter than urea-treated thatch samples. This probably accounted for the differences in urease activity. Nevertheless, these results suggest that the potential for high levels of urease activity existed in thatch prior to urea fertilization.

Incubating thatch that was previously air dried for 2 weeks resulted in a large increase in urease activity over that in wetter samples (Table 1). This extreme elevation in thatch urease activity can be compared to the effects of remoistening air dried soils resulting in a microbiological "burst" of activity (1, 10, 11, 15). In contrast, 2 weeks of air drying soil did not have any affect on urease activity (Table 1). These results reflect the stability of soil urease activity reported by other workers (4, 12, 17). Furthermore, these urease activity levels were comparable to those obtained by other workers for silt loam soils (12, 16).

Table 2. Effects of greenhouse incubation of winter-dormant turf on urease activity within the thatch layer.

Treatment	% Dry wt	Mean ($\bar{x}$) urease activity†
		μg urea-N hydrolyzed g^{-1} sample dry wt h^{-1}
Incubation period		
0 days	64.2	635.7
3 days	38.4	522.6
10 days	48.4	887.4
LSD (0.05)		117.3

† Mean ($\bar{x}$) of eight replications.

Significant seasonal variation in thatch urease activity was apparent in the comparisons between the September 1980 data (Table 1) to the March 1981 data (Table 2). These results are in sharp contrast to the remarkable stability of urease activity found in soils (17). The seasonal effect on thatch urease activity may be due to differences in temperature, moisture level, or by the fact that living turfgrass tissue and/or microbial populations within the thatch were dormant during the March sampling period. Table 2 shows the effects of greenhouse incubating winter-dormant thatch. Thatch samples analyzed directly from the field (0 days) exhibited urease activity levels that were not significantly different than those found after the 3-day greenhouse incubation. Increasing the duration of greenhouse incubation to 10 days significantly increased the level of thatch urease activity. This variable nature of thatch urease activity suggests that levels of protective organic colloidal material are low compared to soil and that the level of thatch urease may depend upon the metabolic activity of both the turfgrass plants and microbial population.

Table 3 shows the variation in urease activity within a thatch profile and between sampling locations taken in March 1981. A higher level of urease activity was found in the upper 1-cm section compared to the lower 1-cm section of a 2-cm thick thatch. Variation in thatch urease activity occurred between sampling sites. This variation was not surprising in view of the heterogenous nature of thatch. The thickness and composition of thatch may vary substantially within turf due to disease occurrence, weed infestation, variation in soil properties, and species composition of the turfgrass area. Vertical section differences in thatch urease activity could possibly be due to the amount of living turfgrass tissue found within sections of the thatch profile. The high levels of urease activity associated with turfgrass tissue (Table 1) support this idea but the microbial populations within the thatch profile must also be considered.

The results of this study showed that urease activities within thatch and associated with turfgrass tissue were extremely high compared to activities in underlying soil. Of secondary importance is the fact that urease activity in thatch has been shown to be quite variable in nature in contrast to the stability of soil urease.

The findings suggest a significant impact of thatch upon the fate of urea nitrogen applied to turf. The high levels of urease activity associated with turfgrass tissue and thatch are evidence in favor of a mechanism proposed by Torello et al. (13) to explain ammonia volatilization from

Table 3. Variability of thatch urease activity between sampling sites and within a thatch profile.

Treatment	% Dry wt	Mean ($\bar{x}$) urease activity†
		μg urea-N hydrolyzed g^{-1} sample dry wt h^{-1}
Thatch profile		
Upper 1 cm	35.5	848.0
Lower 1 cm	43.1	464.3
		*
Sampling site		
Location A	64.2	635.7
Location B	57.0	1017.3
		*

* Significant F value ($P = 0.05$).
† Mean ($\bar{x}$) of eight replications.

turfgrass stands. This mechanism suggests that ammonia volatilization will occur from most turfgrass stands which have been treated with urea, regardless of the type of underlying soil, unless the urea is thoroughly washed into the soil. When urea is spray applied in water or applied dry to turf with a thatch layer, the high urease levels in the thatch could cause rapid hydrolysis of the urea which in turn would increase the pH of the water film on the thatch and turfgrass tissue. The increase in pH would help promote ammonia volatilization.

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