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KURA CLOVER AND BIRDSFOOT TREFOIL RESPONSE TO SOIL pH

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ABSTRACT

Use of the rhizomatous perennial forage legume kura clover (*Trifolium ambiguum* M. Bieb.) has been limited by slow establishment. Mature kura clover responds to liming on some acid soils, but the soil pH required for vigorous growth of young plants is unknown. A factorial greenhouse experiment was conducted with two kura clover cultivars (Rhizo and Endura) and one cultivar of birdsfoot trefoil (*Lotus corniculatus* L., Norcen) planted in three soil types (Sartell loamy fine sand, Hubbard loamy sand, and Sanborg clay loam) amended with $Ca(OH)_2$ to obtain six soil pH levels. The experiment was performed twice, once using soil taken directly from the field and once using steamed soil. Response of kura clover and birdsfoot trefoil to soil pH differed. Maximum yield increases in kura clover obtained by adjusting soil pH from 4.9 to 6.5 were about 50% on nonsteamed

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soil and more than 150% on steamed soil. Birdsfoot trefoil did not respond to liming on nonsteamed soil. On steamed soil birdsfoot trefoil response to liming was inconsistent. Optimal soil pH for growth of kura clover and birdsfoot trefoil was generally between pH 6 and 7. Biomass yield was correlated with nodulation in both kura clover and birdsfoot trefoil, but nodulation was correlated with nitrogen uptake only in kura clover. Increased biomass yield of young kura clover plants in response to liming was best explained by alleviation of aluminum (Al), zinc (Zn), and manganese (Mn) toxicities and increased availability of phosphorus (P) and molybdenum (Mo) at higher soil pH levels.

INTRODUCTION

Kura clover is a rhizomatous perennial forage legume with potential to be a major grazing crop due to its persistence under adverse environmental conditions (1). Major limitations to widespread adoption of kura clover have been low seedling vigor and slow establishment (2). Therefore, much research with the species has been conducted to determine the best management strategies for rapid establishment (3–5). However, the effect of soil pH on kura clover seedling growth has not been studied.

Liming of acid soils has been a traditional strategy to increase crop yields. Poor growth on acid soils is due largely the increased activity or solubility of Al and Mn at low pH levels (6). Both Al and Mn can be directly toxic to plants and decrease uptake of Ca and Mg. Zinc can also reach toxic levels on acid soils (7). In contrast, P and Mo can become less available on acid soils due to their reaction with Al and iron (Fe) (6). Legumes can be especially sensitive to acid soils because the activity of some rhizobia species is reduced when soil pH is less than 6.0 (8).

A pH greater than 7.0 can limit crop yields on some soils. As pH levels increase, the availability of all micronutrients except Mo is reduced (6). Highly weathered acid soils may be particularly susceptible to over-liming. Liming can cause deterioration in the structure of these soils and reduce the availability of some minerals (9).

Birdsfoot trefoil is regarded as one of the more acid tolerant forage legumes (10). The percent yield increase of established kura clover in response to liming was about equal to alfalfa (*Medicago sativa* L.), and 2.5 to 4 times greater than birdsfoot trefoil (11,12). Liming increased forage yield of established "Rhizo" kura clover by 24% on a sandy soil in north central Minnesota (11).

Kura clover is persistent in harsh environments such as on acid soil, but it also responds to liming. A diploid line (CPI 2264) demonstrated persistence in an

acid alpine environment and was superior to a tetraploid line (CPI 6884) (13). Both lines were more persistent than four hexaploid lines and *Trifolium repens* L., *Trifolium hybridum* L., and *Trifolium fragiferum* L. (13). The diploid cultivar Summit and the tetraploid cultivar Treeline persisted much better than *T. repens* on soil with low pH and extracted P and calcium (Ca) more efficiently from acid soils (14).

Little is known about how kura clover will respond to liming on the variety of acid soils present in north central North America. On an acid, brown sandy clay loam Oxisol, low availability of copper (Cu) and Mo limited growth of kura clover (12), but different soils may reveal additional possibilities for deficiencies or toxicities. The goals of this study were to compare kura clover and birdsfoot trefoil responses to soil pH during the establishment phase, and to determine the mineral toxicities and deficiencies that may limit growth of young kura clover plants on acid soils typical of the north central United States. Birdsfoot trefoil was included in the study because of its known tolerance to low pH soils.

MATERIALS AND METHODS

Two cultivars of kura clover, "Rhizo" and "Endura," and one cultivar of birdsfoot trefoil, "Norcen," were used. Rhizo was developed by selection for vigorous rhizomatous growth, persistence, and disease and insect resistance (15). Endura is a recently developed proprietary cultivar (Challenge Seeds, Ltd., New Zealand). Norcen is a nine-clone synthetic that is adapted to the mid-western United States (16).

Three soils were used: Sartell loamy fine sand (mixed, frigid Typic Udipsamment) was collected from a recently cleared forest in Saint Louis Co., MN; Sanborg clay loam (fine, mixed, active, frigid Oxyaquic Glossudalf) was collected from a cropped field in Ashland Co., WI; and Hubbard loamy sand (sandy, mixed, frigid Entic Hapludoll) was collected from a stand of red pines (*Pinus resinosa* Aiton) in Sherburne Co., MN. Soil was collected from the upper 25 cm of the profile at each location. All soils were tested for pH (1:1 soil/water, stirred), organic carbon (C), P (Bray 1), potassium (K), sulfur (S), Zn, Fe, magnesium (Mg), Cu, boron (B), Ca, and Mn and fertilized according to the University of Minnesota soil test recommendation for legumes. The Sartell soil had a pH of 4.6, a SMP buffer index of 6.4, contained 22 g kg^{-1} C, and was fertilized with 150 mg kg^{-1} K. The Sanborg soil had a pH of 4.8, a SMP buffer index of 6.1, contained 13 g kg^{-1} C, and was fertilized with 10 mg kg^{-1} P, 113 mg kg^{-1} K, and 11 mg kg^{-1} S. The Hubbard soil had a pH of 5.0, a SMP buffer index of 6.8, contained 5 g kg^{-1} C, and was fertilized with 8 mg kg^{-1} P, 77 mg kg^{-1} K, 9 mg kg^{-1} S, 0.8 mg kg^{-1} B, and 29 mg kg^{-1} Mg.

Ten 250-g samples were taken from each of the three soils and amended with a range of $Ca(OH)_2$ concentrations. After allowing 14 d for partial equilibration, the pH of each sample was determined, and the results were used to construct a calibration curve for each soil. Using the curves, the levels of $Ca(OH)_2$ required to obtain six roughly equally-spaced pH treatments from the starting pH to pH 7.5 was determined. The Sartell soil was treated with 0, 0.4, 1.2, 2.0, 2.8, and 4.4 g kg^{-1} $Ca(OH)_2$. The Sanborg soil was treated with 0, 0.4, 1.0, 1.6, 3.2, and 6.4 g kg^{-1} $Ca(OH)_2$. The Hubbard soil was treated with 0, 0.2, 0.4, 0.6, 1.0, and 1.4 g kg^{-1} $Ca(OH)_2$. All of the soils were allowed to equilibrate for at least 21 d. The soil pH of every pot was tested before planting and after harvesting, and the averages of the two tests were used in all calculations (pH difference always < 1.0).

Plastic pots 18 cm in diameter and 45 cm tall were filled with about 11 kg of soil, and about 45 seeds were planted in nine equally spaced locations. The kura clover was inoculated with a commercial mixture of *Rhizobium leguminosarum* biovar *trifolii*, and the birdsfoot trefoil was inoculated with a commercial mixture of *Mesorhizobium loti*. After 10 d, the plants were thinned to nine plants per pot. Greenhouse temperature was maintained between 22 and 25°C. Supplemental lighting (high pressure sodium vapor) was provided for 16 h d^{-1} at about 90 μmol s^{-1} m^{-2} photosynthetic photon flux. Plants were watered with deionized water to prevent plant water stress.

The experiment was conducted twice. The first trial was planted on 24 January 1997, and the second trial was planted on 20 September 1997. Before the second trial, the soil was steamed two times for 2 h with 5 d between steamings to reduce soil-borne pathogens. In both trials, three samples of each soil type having low, moderate, and high pH were taken immediately before planting and analyzed for several chemical constituents using standard extractants (University of Minnesota Soil Testing Laboratory), with the exception of Al, which was extracted with DTPA. Because the sampling was not replicated, statistical analysis of the data was not performed (Table 1).

Two harvests were performed when the birdsfoot trefoil was beginning to flower at 56 and 91 d after planting. The kura clover was vegetative at both harvests. At the first harvest, the plants were cut to a stubble height of 2.5 cm. At the second harvest, the plants were dug, and the root portion was separated from the shoot at the crown. After harvesting, the plant material was washed with deionized water, dried at 60°C for 72 h, and weighed.

Nodulation and leaf chlorosis were rated visually on a 1 to 5 scale. Chlorosis was rated before the second harvest on the percentage of leaf area that was chlorotic. Nodulation was rated only for kura grown on the Hubbard soil in the first trial, but all treatments were rated in the second trial. The nodulation rating was based on the number and size of nodules present after the roots had been washed.

Table 1. Soil Chemical Properties of Each Soil Type at Low, Moderately Low, and Near-Neutral Soil pH Levels Before and After Steaming

Treatment	Soil	pH[a]	Bray-P (mg kg^{-1})	Ca[b] (mg kg^{-1})	Mg[b] (mg kg^{-1})	K[b] (mg kg^{-1})	Na[b] (mg kg^{-1})	Fe[c] (mg kg^{-1})	Mn[c] (mg kg^{-1})	Zn[c] (mg kg^{-1})	Cu[c] (mg kg^{-1})	Ni[c] (mg kg^{-1})	Al[c] (mg kg^{-1})
Nonsteamed	Sanborg	4.7	38	1310	231	128	26	169	36	1.4	0.77	0.36	29
		5.4	27	2000	275	135	24	116	20	0.6	0.62	0.22	8
		7.4	43	3910	111	139	15	54	6	0.5	0.55	0.14	1
	Hubbard	5.3	37	310	50	95	9	38	74	0.9	0.24	0.31	29
		5.9	28	504	51	95	9	22	41	0.6	0.24	0.18	13
		7.4	24	811	41	91	6	14	47	0.3	0.24	0.09	4
	Sartell	4.9	114	599	79	229	13	139	32	2.2	0.19	0.20	100
		5.9	108	1140	85	198	24	100	20	1.1	0.16	0.13	31
		7.1	104	2050	66	216	15	55	8	0.9	0.18	0.06	6
Steamed	Sanborg	5.0	28	1390	266	128	37	147	60	1.1	0.71	0.28	13
		5.6	28	1770	235	100	29	111	53	0.9	0.72	0.28	5
		7.1	36	3060	89	112	28	40	19	0.4	0.56	0.16	1
	Hubbard	5.4	32	366	62	68	24	34	115	0.8	0.24	0.07	13
		6.1	24	466	57	41	16	17	75	0.5	0.24	0.26	7
		6.9	26	644	45	41	19	13	30	0.3	0.23	0.10	3
	Sartell	4.9	96	679	101	209	43	132	109	2.2	0.21	0.06	62
		5.7	89	1280	103	156	39	64	77	0.9	0.14	0.22	20
		6.8	86	1960	83	156	31	48	34	0.9	0.18	0.05	5

[a] 1:1 soil/water, stirred.
[b] Extracted with 1 *M* ammonium acetate.
[c] Extracted with DTPA.

The shoot material from the second harvest was ground in a cyclone mill with a 1-mm screen and collected in plastic screw-top bottles. The samples were tumbled in a plastic drum turning at 15 rpm for 20 min to obtain homogeneity. Near infrared reflectance spectra (NIRS) were collected on all samples of sufficient size using a NIRS scanning monochromator, model 6500 (Foss North America, Inc., Eden Prairie, MN 55344) and NIRS version 4.0 software (Infrasoft International, Port Matilda, PA 16870). Reflectance data were recorded between 400 and 2500 nm wavelengths at 2-nm intervals. Laboratory elemental analysis was performed on 53 samples selected by the software to develop an NIRS equation for tissue mineral concentration. The laboratory analysis was conducted using simultaneous multi-element Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES) (17,18). The laboratory values were compared to the values calculated by the NIRS equation, and the NIRS equation adequately predicted tissue concentrations of P, K, Ca, Mg, and Mn ($r^2 > 0.87$). Tissue N content was approximated using a previously developed NIRS equation.

The experiment used a factorial arrangement of treatments with three soil types by three cultivars (two kura clover and one birdsfoot trefoil) by six soil pH levels in a randomized complete block design with three replications. Each experimental unit consisted of a single pot with nine plants. Because soil pH was somewhat different in every pot, regression analysis was used to determine the response of first harvest shoot, second harvest shoot, and root dry matter production to soil pH. Responses of the three yield components were similar, so all further analysis was conducted using total biomass yield (root and both shoot harvests combined). The analysis was begun by fitting a model with separate intercept, linear, and quadratic terms for each treatment combination (soil type and cultivar). Quadratic or linear terms were deleted from the model when their removal resulted in a simplified model that was still adequate when compared to the full model by an F-test ($p > 0.05$). Due to highly significant interactions, the extremely complex model of a separate slope and intercept for every treatment combination could not be simplified. Because soil pH levels were slightly different for every treatment, mean yields could not be computed for specific soil pH levels. Instead, the regression model for each treatment combination was used to calculate predicted yield values at pH 4.9 and 6.5. To examine the relationship between soil mineral concentration and plant yield, regression analysis was performed between total biomass yield and P and Mn tissue concentrations. Tissue concentrations of other minerals were either within acceptable ranges in all samples or not adequately predicted by NIRS equations. All statistical calculations were performed with S-Plus 4.5 (19).

RESULTS AND DISCUSSION

Soil Properties

Amending the soils with $Ca(OH)_2$ effectively produced a range in soil pH from less than 5 to about 7.5 in the Sanborg and Hubbard soils. The Sartell soil ranged from about pH 4.5 to 7. After steaming, the pH of the Hubbard and Sartell soils ranged from about 5 to 7, and the Sanborg soil pH ranged from less than 5 to greater than 7, but there were no treatments between pH 6 and 7.

Many soil chemical properties varied by soil and were apparently altered by liming and steaming (Table 1). In most cases, liming decreased extractable Fe, Mn, Zn, and Al, and consistently increased extractable Ca. Extractable Al and K were substantially decreased after steaming, but extractable Mn increased. At pH <5.5, extractable P was consistently reduced by steaming. The Sartell soil had the highest levels of extractable P, K, and Al at all pH levels.

Yield Response to Soil pH

On nonsteamed soil, kura clover biomass yield was influenced by soil pH in every combination of soil and cultivar except Rhizo grown on the Hubbard soil (Fig. 1). Norcen birdsfoot trefoil, however, did not respond to pH on any of the nonsteamed soils. Increasing the pH of the Sartell soil from 4.9 to 6.5 increased biomass yield of Endura and Rhizo by 52 and 33%, respectively. Increasing the pH of the Hubbard soil from 4.9 to 6.5 increased biomass yield of Endura by 22%. These yield increases were comparable to increases in the yield of established kura clover obtained by liming (11). Liming the nonsteamed Sanborg soil did not increase biomass yield of kura clover.

In the second trial, which used steamed soil, kura clover biomass yield was influenced by soil pH in every combination of soil and cultivar (Fig. 1). Norcen birdsfoot trefoil biomass yield was influenced by pH only on the Sanborg soil. On the Sanborg soil, the yield response of all cultivars to soil pH was quadratic. The quadratic trend was due to very low yields at soil pH > 7. This yield reduction was likely associated with a loss of soil structure similar to that documented for Ultisols and Oxisols (9). Over-liming of clay soils like Sanborg is expensive given the high lime requirement and may be detrimental. Increasing pH of the Sanborg soil from 4.9 to 6.5 increased biomass yield of Endura, Rhizo, and Norcen by 135, 88, and 38%, respectively. Increasing soil pH from 4.9 to 6.5 increased biomass yield of Endura and Rhizo on Hubbard soil by 74 and 105%, respectively, and on Sartell soil by 169 and 104%, respectively.

Biomass yields were substantially lower in the second trial with steamed soil than in the first trial with nonsteamed soil (Fig. 1). This effect may be

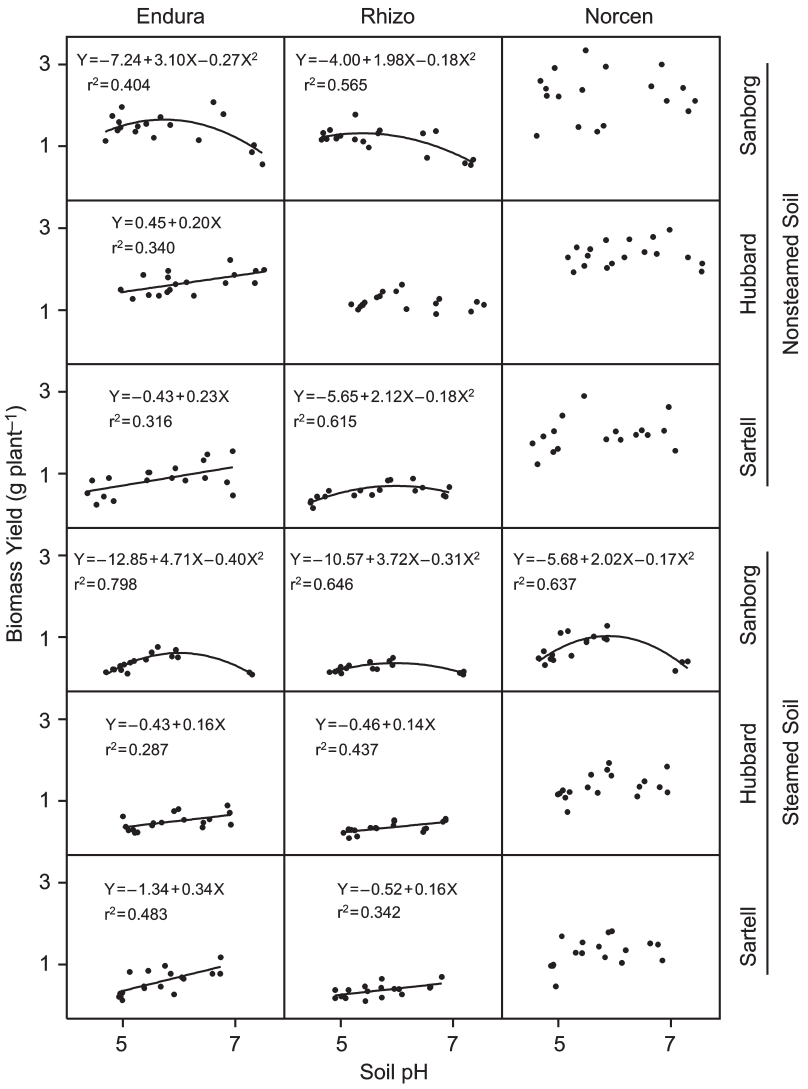

Figure 1. Relationship between soil pH and biomass yield (root and two shoot harvests) of Endura and Rhizo kura clover and Norcen birdsfoot trefoil. Regression lines are significant at $p < 0.05$.

partially due to day length or other environmental differences between the two trials, but soil steaming is known to have a detrimental effect on growth of legume seedlings (20).

Plant Tissue Mineral Concentrations

Minimum and maximum mineral concentrations were compared to the critical and toxic levels of these minerals for *Trifolium repens* L., *Trifolium pratense* L., and *Trifolium subterraneum* L. (Table 2). Tissue K, Ca, Mg, Fe, Cu, and B were within acceptable levels in the kura clover cultivars, but both Mn and Zn exceeded potentially toxic levels in some treatments at low pH. Molybdenum fell below the level of detection on low pH soils and was potentially limiting to growth. Tissue concentration of P was below potentially critical levels in some acidic soil treatments. Unfortunately, Zn and Mo tissue concentrations were not adequately predicted with NIRS and were not investigated further.

Tissue Mn concentration was negatively correlated with biomass yield in 7 of the 12 kura clover/soil type combinations (Fig. 2) but was not correlated with biomass yield of birdsfoot trefoil. Tissue Mn concentration was negatively correlated ($p < 0.001$) with soil pH in all cultivars on all soils except nonsteamed Sanborg. The correlation between soil pH, tissue Mn concentration, and biomass yield suggest Mn toxicity as a possible mechanism for limiting yield of kura clover at low pH on the soils used in this study.

Tissue P was correlated with biomass yield of Endura grown on steamed Sanborg soil and Rhizo grown on steamed Sanborg and Hubbard soils (Fig. 3). Because the relationship was present only on steamed soil, low P uptake is not likely to be a common mechanism by which kura clover yields are limited at low pH in these soils under field conditions. None of the soils used had particularly low extractable P concentrations because we added P to reduce the likelihood of deficiency.

Visual Ratings

The plants ranged from showing no chlorosis (rating = 1) to 20% of the leaf area being chlorotic (rating = 5). In the extreme cases, necrosis was also beginning in small spots. In the soil by cultivar treatments in which tissue Mn concentration was negatively correlated with biomass yield (Fig. 2), tissue Mn concentration was also correlated with chlorosis rating. Foliar chlorosis in kura clover was moderately correlated with tissue Mn concentration on nonsteamed soil (slope = 0.0021, $r^2 = 0.329$, $p < 0.001$) and well correlated

Table 2. Minimum and Maximum Mineral Concentrations of ICP-AES Tissue Analysis with Corresponding Critical/Marginal and Toxic Levels

	P ($mg\,kg^{-1}$)	K ($mg\,kg^{-1}$)	Ca ($mg\,kg^{-1}$)	Mg ($mg\,kg^{-1}$)	Mn ($mg\,kg^{-1}$)	Fe ($mg\,kg^{-1}$)	Zn ($mg\,kg^{-1}$)	Cu ($mg\,kg^{-1}$)	B ($mg\,kg^{-1}$)	Mo ($mg\,kg^{-1}$)
Minimum										
Endura	1920	21,500	18,400	3270	47	112.4	13.8	6.5	21.2	<0.22
Rhizo	2110	22,900	18,700	3090	60	93.1	16.4	8.3	32.8	<0.22
Norcen	1540	21,800	8,320	2690	41	64.8	15.5	5.3	13.1	<0.22
Maximum										
Endura	3560	31,600	35,200	7130	1600	339	120	18.8	89.3	7.62
Rhizo	3950	29,200	34,300	5380	1880	461	151	19.6	101.8	5.83
Norcen	3490	41,300	31,000	5970	1780	298	120	20.7	85.4	8.87
Critical/Marginal[a]	2500–3000	8000–12,000	9000–11,000	1800–2500	25.0	50.0	15.0	4–5	13–16	0.15
Toxic[b]	—	—	—	—	600–800	—	50	—	250	—

[a] Values are either marginal or critical levels for *T. repens* as given by Reuter and Robinson (21).

[b] Mn toxic level is for *T. repens*, Zn toxic level is for *T. subterraneum*, B toxic level is for *T. pratense*, others were not available; values are from Reuter and Robinson (21).

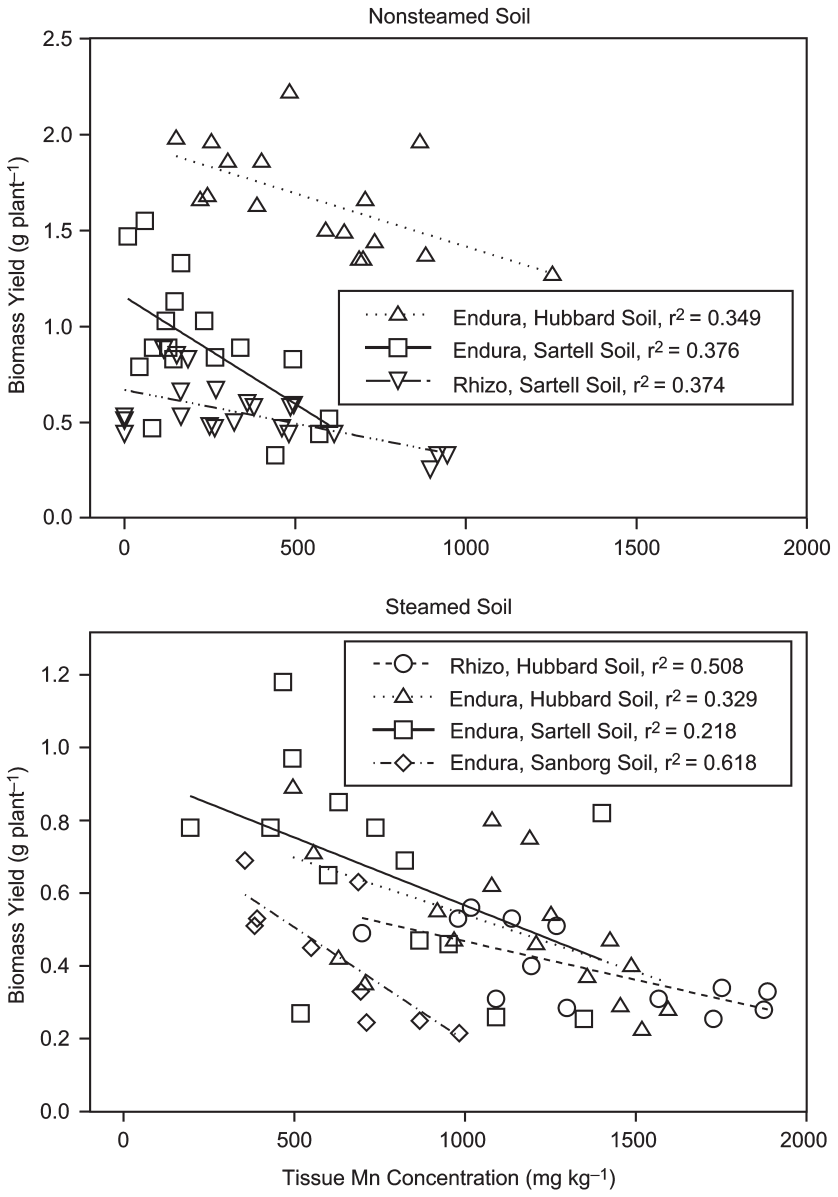

Figure 2. Relationship between tissue Mn concentration and biomass yield (root and two shoot harvests) of kura clover. Regression lines are significant at $p < 0.10$.

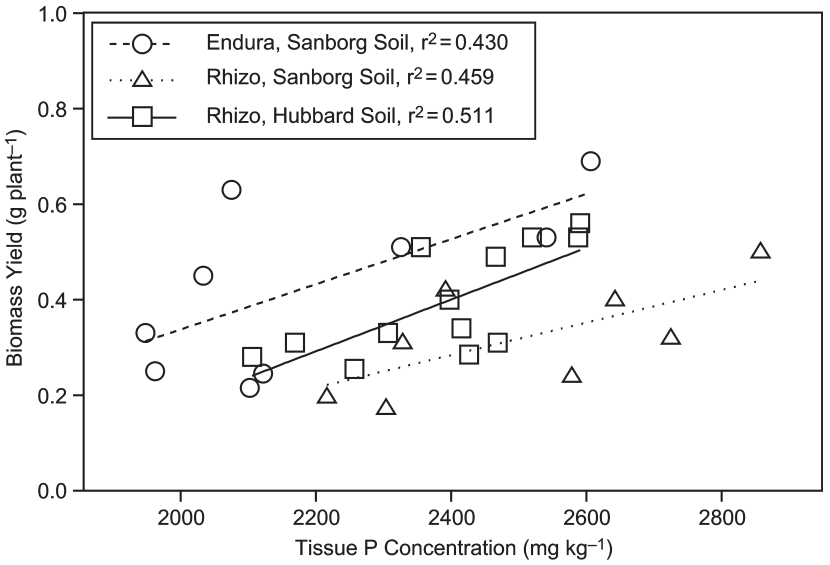

Figure 3. Relationship between tissue P concentration and biomass yield (root and two shoot harvests) of kura clover grown on steamed soil. Regression lines are significant at $p < 0.10$.

with tissue Mn concentration on steamed soil (slope = 0.0023, $r^2 = 0.656$, $p < 0.001$). The relationship between tissue Mn concentration and leaf chlorosis shows that chlorosis is a good indicator of Mn toxicity in kura clover. The chlorosis occurred primarily at the leaf margins, which is a typical Mn toxicity symptom (21). Leaf chlorosis was not observed on Norcen in any soil treatment, suggesting that it is probably tolerant of high foliar concentrations of Mn, or that chlorosis is not a typical symptom of this condition (22).

The plants ranged from having no nodules (rating = 1) to being heavily nodulated (rating = 5). In the first trial, nodulation was only rated for the kura clover cultivars on the Hubbard soil, because high clay and organic matter content made washing difficult on the other soils. Both kura cultivars had significant linear increases in nodule rating with increasing soil pH (slope = 0.899, $r^2 = 0.356$, $p < 0.001$). In the second trial, nodulation was rated for all cultivars on all soils. No responses were seen on the Sanborg soil. On the Hubbard soil, Endura (slope = 0.964) and Norcen (slope = 0.790) had significant linear increases in nodule rating with increasing pH ($r^2 = 0.633$, $p < 0.001$). On the Sartell soil, both kura clover cultivars had significant linear

increases in nodule rating with increasing pH (slope = 0.357, $r^2 = 0.287$, $p < 0.001$).

Across all soils, total biomass yield of kura clover (slope = $0.767X - 0.090X^2$, $r^2 = 0.499$, $p < 0.001$) and birdsfoot trefoil (slope = 0.211, $r^2 = 0.407$, $p < 0.001$) increased with increasing nodule rating. Nitrogen uptake (g plant^{-1}) also increased with increasing nodule rating in kura clover (slope = $0.010X - 0.001X^2$, $r^2 = 0.421$, $p < 0.001$). In birdsfoot trefoil, N uptake was not correlated with nodule rating. Therefore, reduced nodulation of kura clover at low soil pH probably limited plant growth through a reduction of biological N_2 fixation, but reduced nodulation of birdsfoot trefoil did not affect plant growth. This result is consistent with the finding that kura clover seedling growth is highly dependent upon nitrogen availability (4). Birdsfoot trefoil may be more capable of taking up soil N in acid soils that limit nodulation and biological N_2 fixation.

CONCLUSIONS

Kura clover was more consistently responsive to increased soil pH than Norcen birdsfoot trefoil during establishment. Biomass yield increases of up to 52% in kura clover were obtained by increasing the pH of nonsteamed soil from 4.9 to 6.5. Increases in young plant growth of this magnitude justify liming sandy soils similar to Hubbard or Sartell to pH > 6.0 before establishing kura clover. Liming of the Sanborg soil is not recommended because increasing pH of this soil in the nonsteamed trial produced little positive impact on plant growth. Field studies are needed to verify these results and determine economically optimum pH levels.

Increases in biomass yields of kura clover on soils such as those used in this study are most likely due to alleviation of Al, Mn, or Zn toxicities, and in some cases, increased availability of P or Mo. Extractable Al decreased with liming, and legume seedlings are known to be sensitive to Al (23). All low-pH nonsteamed soils had extractable Al concentrations higher than the concentration (DTPA–Al = 27 mg kg^{-1}) suspected of causing injury to birdsfoot trefoil roots (24). Foliar chlorosis and reduced biomass yield were correlated with tissue Mn concentration in some kura clover treatments, but not in birdsfoot trefoil. Some plant species are tolerant of high Mn concentrations in their foliage (7,25); our results indicate that birdsfoot trefoil may be a tolerant species.

Root systems of kura clover grown on sandy soils generally showed evidence of increased nodule size and abundance in response to increased soil pH, demonstrating that the growth or activity of the rhizobia is inhibited by low soil pH. On acid soils, liming will probably be required to obtain maximum levels of N_2 fixation in kura clover.

ACKNOWLEDGMENTS

Names are necessary to report factually on available data, however, the USDA and the University of Minnesota neither guarantee nor warrant the standard of the product, and the use of the name by the USDA and the University of Minnesota implies no approval of the product to the exclusion of others that may be suitable.

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