Subterranean Clover Versus Nitrogen Fertilized Annual Grasslands: Botanical Composition and Protein Content[1]

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Highlight

Application of N increased the percentage grass and depressed annual legumes. Subterranean clover not N fertilized was very competitive with grass when the sward was grazed or mowed. The establishment of subterranean clover resulted in higher protein forage when need was greatest during the dry season, compared with N fertilization which depressed protein levels during the same period.

Nitrogen fertilization of California's annual grasslands generally has produced a marked increase in production of forage. The effect of applied N on botanical composition and on protein level in the plants is also of interest on these extensive grasslands. Hoglund et al. (1952) indicated that neither nitrogen nor phosphorus had any important effect upon botanical composition where plots were clipped. However, Evans and Love (1956) indicated that annual grassland species such as soft chess *(Bromus mollis)*, ripgut brome *(B. rigidus)*, and wild species of oat *(Avena fatua* and *A. barbata)*, and broadleaf filaree *(Erodium botrys)* increased in percentage when nitrogen, especially in combination with phosphorus and sulfur, was applied. Jones and Evans (1960) studied the effect of applied nitrogen on botanical composition of annual grasslands. They concluded that the species reacted differently to fertilization under grazing than where no grazing occurred. However, the percentage of clover was reduced where nitrogen was applied, whether the plots were grazed or not. Subterranean clover *(Trifolium subterraneum)* was reduced much more on ungrazed plots than on grazed plots by the application of nitrogen. Davies et al. (1966) fertilized plots in a pasture of soft chess and subclover with three levels of ammonium sulfate (0, 250, or 700 lb/acre at emergence and in late winter for 3 years. Each plot was grazed continually with Merino sheep at two stocking rates: 3.5 and 5.0 sheep/acre. The heaviest rate of N eliminated clover from the pasture at both stocking rates by the second year. At the intermediate nitrogen rate, clover was reduced more with light stocking than with heavy stocking. Jones et al. (1961) measured botanical composition on N-fertilized and unfertilized range at 11 locations in the California annual type grasslands. Annual grasses and filaree increased on the fertilized pastures. The percentage of legumes was reduced when N was applied the same season of sampling; but where N had been applied only in years previous to the season of sampling, the percentage of ground covered by legumes was not different from that on the unfertilized pastures. Woolfolk and Duncan (1962) also reported that applications of N increased percentage of grass in the total forage.

Due in part to changes in botanical composition and to physiological changes within the plants themselves, N fertilization has important effects on the protein levels in forage from annual grassland pastures. Hoglund et al. (1952) reported that percent protein at maturity was slightly decreased where N was applied at 84 lb/acre. Jones (1960) reported that the effect of N fertilizer on the protein level in the plant varied with the date N was applied. When N was applied early in the fall, the percent in the plant increased during the winter and spring but decreased at maturity, compared with the unfertilized grass. When N was applied in the spring, the percent N in the mature plants was higher than in the unfertilized plants. In another study, Jones (1963) followed the protein level in individual species through the season after various levels of N had been applied in the fall. All rates of N increased the protein level early in the season. As the plants matured, the percent protein in the nonleguminous species usually was decreased with the addition of 40 lb N/acre. Apparently N stimulated growth to such an extent that N levels in the plant were more dilute. By the end of the growing season the decrease in forage production by clover species had resulted in a decrease in the protein level in the total forage. Plants fertilized with 80 lb N/acre generally had concentrations of protein equivalent to those of unfertilized plants; 160 lb N generally increased percent protein in the nonleguminous plants. McKell and Graham (1960) reported that increasing rates of N fertilization increased the protein level in mature plants grown in a 10-inch rainfall zone. It seems apparent that under their conditions moisture was limiting for plant growth, and thus protein levels were not diluted in the plant tissues. In the study by Davies et al. (1966) samples of

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soft chess and subterranean clover taken late in the fall had concentrations of 2.6 and 11.8% protein, respectively. The variation in protein level in the two species illustrates the importance of changes in botanical composition on protein levels in annual pasture species. Sheep produced more wool and meat on pastures high in subterranean clover than on pastures where heavy amounts of nitrogen had reduced the pasture to grass species.

The purpose of the present experiment was to compare changes in botanical composition and protein levels of plants on an area fertilized with N with similar changes where subterranean clover was established.

Table 1. Botanical composition (percent[1]) of N-fertilized resident grassland and unfertilized subterranean clover sward under three types of management on a Sutherlin loam site.

Management	Subterranean clover sward	Resident grassland (1b N/A)			
		0	40	80	160
Grasses					
Grazed	18[a]	41[cd]	49[de]	73[ghi]	81[hi]
Mowed	17[a]	29[b]	39[bcd]	63[fg]	71[gh]
Ungrazed-unmowed	34[b]	36[bc]	53[ef]	69[g]	83[i]
Forbs					
Grazed	9[ab]	24[def]	26[f]	16[bcde]	13[abc]
Mowed	6[a]	19[cdef]	28[f]	21[cdef]	25[ef]
Ungrazed-unmowed	8[ab]	15[abcd]	14[abc]	16[bcde]	14[abc]
Annual clover					
Grazed	16[c]	35[e]	25[d]	11[bc]	6[ab]
Mowed	11[bc]	52[f]	33[e]	16[c]	4[ab]
Ungrazed-unmowed	30[e]	49[f]	33[e]	15[c]	3[a]
Subterranean clover					
Grazed	57[b]	.....	.....	.....	.....
Mowed	66[b]	.....	.....	.....	.....
Ungrazed-unmowed	28[a]	.....	.....	.....	.....

[1] Within each species group, percentages having the same letter in the superscript do not differ significantly at the 10% level.

Procedure

This three-year study was made on two soils—Sutherlin loam (resident annual grassland) and Willits loam (hardinggrass *(Phalaris tuberosa)* stand) —on the University of California's Hopland Field Station. Both plot areas were fertilized uniformly with 1,000 lb/acre singlesuperphosphate in October, 1960, and 500 lb/acre singlesuperphosphate in October, 1961 and 1962. There were three main plot treatments in a split-plot design: grazing, mowing, and ungrazed-unmowed. The main plots in each of the four replications were divided into eight subplots treated as follows: (1) check, (2) subterranean clover seeded in October, 1960, which resulted in a good stand of subterranean clover mixed with grasses during the three years of the study; (3), (4), and (5) 40, 80, and 160 lb N/acre applied in October, 1960, and again in October, 1962; (6), (7), and (8) 40, 80, and 160 lb N/acre applied in October, 1961. The nitrogen was applied as urea. Size of individual subplots was 10 x 20 ft.

Plots were sampled three times during the growing season as indicated in the figures of protein values. At each sampling date in the grazed treatments eight or ten sheep were grazed for two or three days until most of the forage had been removed. It was the objective to graze the plots down to about the same level as the mowed plots. The sheep were kept in a corral during the night so that deposits of excretion on the plots would be minimized. Fecal material that was dropped on the plots was removed after each grazing. The mowed treatments were clipped at about 1 inch with a rotary mower at each sampling date. The ungrazed-unmowed plots were mowed only at the end of the season when the plants had matured and dried.

During each summer all plots were mowed and cleared of remaining dry forage. Percent protein in the forage was determined by the Kjeldahl method. Data were analyzed by means of IBM computer. Botanical composition was determined by visually estimating percentages of grasses, forbs, and clovers in three 1-square-foot quadrats per plot at each of the three sampling dates in each of the three years. Occasionally clipped samples were sorted and dry weights were taken to check estimates. Since changes in botanical composition due to treatment were relatively the same at each sampling date only the data collected in May are reported in this paper. The values reported in Tables 1 and 2 are the means of 36 estimates (three quadrats per plot, four replications, and three years). Measurements given in the tables and figures were made during the growing season immediately following the October application of N. More details on measuring yields and N uptake are given in another paper (Jones, 1966).

Results and Discussion

Botanical composition.—Botanical composition at the two sites, as affected by N fertilization or subclover, is given in Tables 1 and 2. Composition was significantly different each year, but the changes due to treatment were in the same direction. Therefore, treatment means averaged over the three-year experimental period are given. There was no consistent difference in plots fertilized 1960 and 1962 compared with those fertilized only in 1961. There was no significant effect from N in the second year after application.

The major annual grasses in the resident pasture (Sutherlin loam site) were soft chess and ripgut brome. Other grasses were foxtail fescue *(Festuca megalura),* annual blue grass *(Poa annua),* medusahead *(Elymus caput-medusae),* and slen-

der oat *(Avena barbata)*. The percentage of these annual grasses increased with increasing rates of N regardless of management. However, there was a tendency for the percentage of grass to be less in the mowed plots than in the two other management treatments. Total forage yield data indicated no significant difference between mowing and grazing (Jones, 1966). There was no significant nitrogen-management interaction in the percent grass data. The percentage of grass on the ungrazed-unmowed plots seeded to subterranean clover was about double that on the subterranean clover plots that were mowed or grazed. Obviously the subterranean clover was much less competitive where the grasses grew unchecked throughout the growing season.

The forbs consisted of filaree *(Erodium botrys)*; fiddleneck, *(Amsinckia sp.)*, and a few others. The percentage of such herbs was not consistently affected by the N treatments except on the grazed plots, where the percentages decreased at the higher levels of N. Percentages of broadleaf herbs were at their lowest levels on the subterranean clover plots.

The percentage and yield (Jones, 1966) of annual clovers decreased consistently with increasing levels of N. The annual clovers consisted of California burclover *(Medicago hispida)*, and native clovers such as *T. ciliolatum*, *T. microcephalum*, and *T. variegatum*. There was no significant difference in annual clover percentages among management treatments except where no N was applied. On the check plot, grazing reduced the amount of annual clover compared to the mowed or the ungrazed-unmowed treatments. This was a reflection of the reduction in California burclover. Where subterranean clover was growing, the annual clover species were at about the same level as where 80 lb N/acre had been applied except in the ungrazed-unmowed management treatment. The increase in the percentage of annual clover on this treatment was primarily a reflection of the increased growth of California burclover in the ungrazed treatment.

Table 2. Botanical composition (percent[1]) of N-fertilized hardinggrass stand and unfertilized subterranean clover sward under three types of management on a Willits loam site.

Management	Subterranean clover sward	Hardinggrass stand (lb N/A)			
		0	40	80	160
Hardinggrass					
Grazed	50^{ab}	61^{cdef}	48^{a}	53^{abc}	68^{ef}
Mowed	54^{abc}	66^{def}	59^{be}	58^{bcd}	70^{f}
Ungrazed-unmowed	57^{ad}	59^{bcde}	51^{abc}	50^{ab}	63^{def}
Annual grasses					
Grazed	9^{a}	22^{b}	36^{dc}	39^{de}	32^{cd}
Mowed	8^{a}	26^{bc}	33^{cd}	36^{de}	27^{bc}
Ungrazed-unmowed	9^{a}	22^{b}	33^{cd}	44^{e}	37^{de}
Legumes and broadleaf herbs					
Grazed	2	17	16	8	0
Mowed	0	8	8	6	3
Ungrazed-unmowed	8	19	16	6	0
Subterranean clover					
Grazed	39^{b}	.....	.....	.....	.....
Mowed	38^{b}	.....	.....	.....	.....
Ungrazed-unmowed	26^{a}	.....	.....	.....	.....

[1]Within each species group, percentages having the same letter in the superscript do not differ significantly at the 5% level. No statistical analysis was run on the legume and broadleaf herbs group because of the high variation between years, there being practically none in this category in the first and third year of the experiment.

Percentages of subterranean clover were highest on the grazed and mowed treatments. These data indicate that the growing habit of subterranean clover is well adapted to grazing during the growing season. In fact subterranean clover must be grazed to maintain a stand competitive with the other grassland species.

On the hardinggrass plots (Willits loam site) the application of 40 lb N/acre consistently decreased the percentage of hardinggrass, the result primarily of an increase in percentage of annual grasses. The percentage of hardinggrass where 80 lb N were applied was similar to that with the 40 lb rate. The highest percentage of hardinggrass was obtained with 160 lb N. In the subterranean clover plots the percentage of hardinggrass was about equal to that in the 40 lb N treatment.

The annual grass at the Willits loam site was mainly ryegrass *(Lolium multiflorum)*. Increasing the level of N up to 80 lb/acre increased the percentage of annual grass, but the percentage of ryegrass was less at the 160-lb N level than at the 80-lb level. The percentage of annual grass on the subterranean clover plots was lower than where no N had been applied.

The major legume at the Willits site was lupine *(Lupinus* sp.) Broadleaf herbs also were included with this category because there were very few except in the mowed treatment. Mowing greatly reduced the percentage of lupine. Application of N also reduced this species.

In the present study changes in botanical composition result-

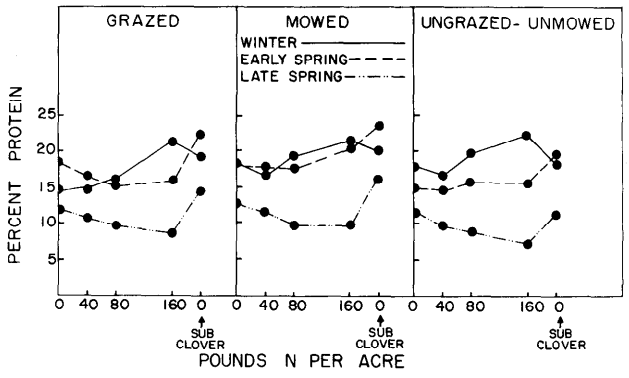

FIG. 1. On a Sutherlin loam, the percentage of protein in forages from grazed, mowed, and ungrazed-unmowed plots at different seasons of the year, as affected by increasing rates of N or seeding of subterranean clover. L.S.D. (.05) = 2.4% protein.

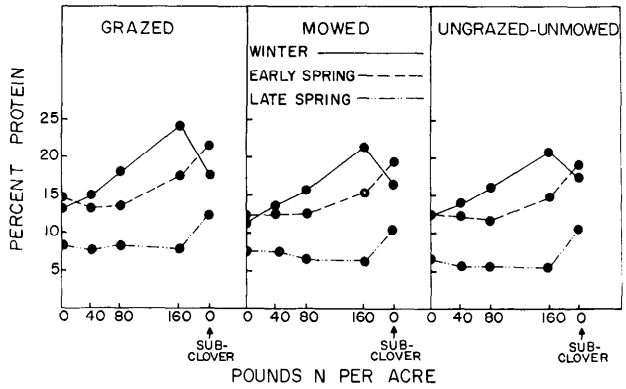

FIG. 2. On a Willits loam, the percentage of protein in forage from grazed, mowed, and ungrazed-unmowed plots at different seasons of the year, as affected by increasing rates of N or seeding of subterranean clover. L.S.D. (.05) = 1.4% protein.

ing from N applications generally appeared to be independent of management treatment except for broadleaf herbs. This observation does not necessarily contradict the findings reported earlier by Jones and Evans (1960). In that study individual grass species were delineated, and the relationship among percentages of these species was found to differ under grazing as compared with no grazing. In this study all the grasses were grouped together. As a group the grasses increased with increasing N under all three management treatments. Of particular interest was the relationship between the legumes and the nonlegumes. These relationships changed but little under the various management systems, with the exception that burclover was consistently less abundant on grazed plots than on mowed or ungrazed-unmowed plots, and subterranean clover was less abundant on ungrazed-unmowed plots.

Protein levels.—Protein levels in the forage from the Sutherlin loam site are given in Fig. 1. The levels generally increased with increasing rates of N during the winter period. The level of protein in the forage from subterranean clover plots was greater than from plots where 80 lb N had been applied, but less than where 160 lb N had been added.

The levels of protein remained high in the spring, but the response to fertilization was somewhat different than in the winter period. On the grazed plots the application of N actually decreased the level of protein, as compared with the unfertilized plots, and the subterranean clover plots had the highest level of protein of any treatment. On the mowed and ungrazed-unmowed treatments, N had little effect upon the protein level except where 160 lb/acre was applied to the mowed plots. But in each instance the subterranean clover plots had the highest level of protein.

At the last sampling date, when plants were approaching maturity, the effect of N on each of the management treatments was somewhat similar. Application of N decreased the level of protein, and the subterranean clover plots had the highest level. Forage from the ungrazed-unmowed plots was consistently lower in N than forage from the other management treatments. This was true for all levels of N and the subterranean clover treatment.

On the Willits soil the response to N fertilization at the first sampling date in late winter or early spring was consistent for all management treatments. Each increment of N increased the level of protein in the forage; and forage from the subterranean clover plots was somewhat lower in protein than that from plots where the highest rate of N had been applied. As the season advanced the effect of the lower rates of N on protein level of the plants became insignificant while the level in the subterranean clover plots was relatively high. At the final sampling date there was no effect from N even at the highest rate and subterranean clover forage had the highest level of protein. Where N had been applied, the ungrazed-unmowed plots were lower in protein than the other management plots. Forage from mowed plots had less protein than forage from grazed plots.

Summary and Conclusions

The effect of increasing levels of N applied to nonirrigated California grasslands was compared to swards of subterranean clover-grass under three types of management: grazed, mowed, and ungrazed-unmowed. Establishment of a subterranean clover stand with resident annual grassland species, or in a stand of hardinggrass, reduced the percentage of all species present other than subterranean clover in each of the three types of management except on the

ungrazed-unmowed treatment where California burclover percentage remained high. Subterranean clover was most competitive on grazed or mowed plots. But clover percentages were reduced by grazing.

Application of increasing rates of N increased the percentage of annual grasses and decreased that of native legumes in the three management treatments. The percentage of hardinggrass did not consistently increase except when the highest rate of N was applied.

Protein levels in forage from subterranean clover plots were as high or higher during the winter period as where 80 lb N/acre had been applied. As the season advanced, protein levels remained high longer on subterranean clover plots, and did not drop to such low levels as where N had been applied.

Fall-applied N increased the protein percentage in the forage during the winter and early spring. At plant maturity, the percentage of protein in the forage was lower where N had been applied than where it had not. As indicated in previous studies, this resulted from a decrease in the percentage of legumes and also from a decrease in the level of protein in the grass species. In areas with less rainfall, the addition of N to annual grasslands may increase the protein level, because all of the applied N is not utilized in plant growth. But in the higher rainfall areas of the state, protein levels are likely to be decreased at plant maturity by the application of N the preceding autumn.

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