THE INFLUENCE OF MANAGEMENT PRACTICES ON MICROBIAL AND TOTAL SOIL NITROGEN[1]

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Abstract: Nitrogen (N) is usually the nutrient most limiting production in semiarid ecosystems and at very low concentrations can seriously impact ecosystem processes. Soil from five mines, incorporating a number of commonly used land reclamation practices (grazing vs. un-grazed; stockpiled vs. direct hauled soil; shrub mosaic vs. grass seed mix; and stubble mulch vs. hay mulch), were sampled and analyzed for soil total N (TN) and microbial biomass N (MBN). All mines were located in semiarid Wyoming in either mixed-grass or sagebrush steppe ecosystems. The various management practices investigated appeared to have little influence on TN. Reclaimed soils averaged 30% less TN than undisturbed native soils, suggesting that N could potentially limit vegetation production. Only two reclaimed sites (grass and shrub) at Mine 1 contained a greater mass of TN than an undisturbed site, and while the reason is unclear, greater precipitation (20% higher relative to the other sites sampled) may be responsible. The microbial communities present in undisturbed soils appear to uptake N more efficiently than microbial communities present in reclaimed soil, relative to total soil N. As N fertilizer is only rarely used in Wyoming surface mines, N can only accumulate in a reclaimed soil via wet or dry deposition or by N-fixation by free-living micro-organisms or through symbiotic relationships. However, as legumes are typically only a small component of the vegetation, presumably deposition and/or microbial fixation of N are responsible for the majority of N accumulation in these ecosystems. Despite the low TN in reclaimed soils, high plant production on these reclaimed soils suggests that TN is not limiting production.

Additional Key Words: semiarid, surface coal mines, nutrient cycling, Wyoming.

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Introduction

Plant growth is often low in arid and semi-arid ecosystems for multiple reasons, including low precipitation, infertile soils, and environmental extremes. Nitrogen (N) is generally the most limiting nutrient to growth in arid- and semiarid ecosystems (Charley and Cowling, 1968; Paul and Clark, 1996). The reasons for low soil N in prairie ecosystems include low precipitation (Jenny, 1930) and a lack of native leguminous species (that actually fix N) which when combined with potentially high rates of gaseous loss (via denitrification and volatilization) and erosional losses (from wind and water), result in a low availability of N. Other means by which N can be lost (leaching, fire) are unlikely to be major factors in many western rangelands due to low precipitation and biomass production.

In the process of mining and reclamation, N is potentially lost from soil due to the disintegration of soil aggregates leading to greater N-mineralization and an increase in inorganic N, i.e., NO_3 and NH_4. These forms of inorganic N are potential substrates for denitrification and volatilization. Another avenue by which N may be lost is due to spillage of soil during the mining and reclamation processes. Though mine operators are required to plant a cover crop on stockpiles of soil, it is likely that an appreciable amount of soil is lost nonetheless due to wind erosion while the stockpile is being built and before the seeded plant community becomes established. While not lost *per se*, the mixing of soil during mining and reclamation processes means that N also becomes diluted.

The objective of our research was to investigate whether a number of management practices commonly used in reclaiming coal mines in Wyoming differed in their ability to maximize soil TN after a minimum period of 10 years after reclamation. In addition, because of the important role microbes play in decomposition, N-mineralization and storage, we also investigated the amount of N present in microbial biomass.

Materials and Methods

Study sites

All mines were located in semiarid regions of southern and eastern Wyoming with varying elevation and precipitation (Table 1). Prior to disturbance, soils were classified as fine-loamy, mixed, mesic Ustic Haplargids at Mines 2 and 4 (Westerman and Prink, 2004) as well as at Mines 1 and 5 (Munn and Arneson, 1999). At mine 3, soils were mapped as coarse-loamy, mixed, mesic Ustic Haplargids (Munn and Arneson, 1999). Reclamation sites had been seeded with native grasses, shrubs, and forbs in an attempt to re-establish plant communities similar to those found prior to disturbance. Undisturbed sites were dominated by the shrubs *Artemisia tridentata* Nutt. ssp. *wyomingensis* Beetle & Young (Wyoming big sagebrush), *Artemesia cana* Pursh (Silver sagebrush), and *Ericameria nauseos* (Pallas ex Pursh) Nesom & Baird (Rubber rabbitbrush) with an understory of grasses. These included *Pascopyrum smithii* (Rydb.) A. Löve (western wheatgrass), *Hesperostipa comata* (Trin. & Rupr.) Barkworth (needleandthread), *Koeleria macrantha* (Ledeb.) J.A. Schultes (prairie junegrass), and *Bouteloua gracilis* (Willd. Ex. Kunth) Lag ex. Griffiths (blue grama).

Table 1. Location, vegetation, elevation and climate (MAP, Mean annual precipitation; MAT, Mean annual temperature) for a series of surface coal mines located in southern and eastern located in Wyoming.

Mine	Latitude / Longitude	Vegetation type	Elevation (m)	MAP[1] (mm)	MAT[1] (ºC)	Ave. frost free period[3] (days)
Mine 1	N 43º2' W 105º50'	Northern mixed-grass prairie	1,646	305	8.7	130
Mine 2	N 43º42 105º14'	Northern mixed-grass prairie	1,371	299[2]	6.7	117
Mine 3	N 41º44' W 108º47'	Shortgrass sagebrush steppe	1,220	216	6.7	110
Mine 4	N 44º10' W 105º27'	Northern mixed-grass prairie	1,375	390	6.7	117
Mine 5	N 41º51' W 106º45'	Shortgrass sagebrush steppe	2,067	323	6.3	118

[1]Western Region Climate Center, 2004
[2]Long-term (20 years) precipitation records from the mine
[3]National Climatic Data Center (Koss et. al, 1988). Frost free days are calculated based on a 90% probability of the minimal temperature being greater than -2 C.

Experimental design

Comparisons of reclaimed soils on surface mined lands are inherently complicated by a lack of reclamation histories, differences in parent material and overburden and changes in regulatory requirements over the years. With this in mind, every attempt was made to find sites in which all variables were similar between comparisons except the compared management practices.

For three of the four commonly used reclamation practices (grazing vs. ungrazed; stockpiled vs. direct hauled soil; shrub vs. grass seed mix) we sampled soils from a site at each of two different surface mines. A suitable second site for comparison of the mulching practices (stubble vs. hay mulch) could not be located. Reclamation practices were paired by age of reclamation and topsoil depth (Table 2). At each mine an undisturbed soil was sampled to serve as reference point.

At all sites (reclaimed and undisturbed), three 100 m transects (replicates) were randomly established and subsamples were taken at four sampling points were located along each transects. At each sampling point, soil samples were taken at depth increments of 0-5, 5-15 and 15-30 cm. In addition, at two points along each of the transects, soil cores of a known volume were excavated at 0-5, 5-15 and 15-30 cm depth increments for determination of bulk density (Blake and Hartge, 1986). These were used to calculate N mass on a per hectare basis.

Laboratory methods

After returning from the field soil samples were sieved to < 2 mm, air-dried and stored at 4˚C. All soils were analyzed for pH (Thomas, 1996); electrical conductivity (EC) (Rhoades, 1996) and soil texture (Gee and Or, 2002) to determine basic soil physiochemical characteristics. After a sub-sample of soil was ground overnight on a roller-grinder, total carbon (C) and N were analyzed using an elemental analyzer (NA 2100 Protein, CE instruments, Italy). Inorganic C (IC) was analyzed by means of a modified pressure calcimeter method (Sherrod et al., 2002).

Soil organic C (SOC) was calculated as the difference between total C and inorganic C (IC). Microbial biomass C (MBC) was quantified by the chloroform fumigation extraction method (Vance et al., 1987) after soils were wet-up to field capacity (Forster, 1995) and microbial biomass N (MBN) was then quantified using a persulfate oxidation method (Cabrera and Beare, 1993). All values are expressed on a dry weight basis

Differences in soil parameters between practices as well as between reclaimed and undisturbed soil were undertaken using a two-way analysis of variance (JMP ver. 4.0.4, SAS, NC) using an $\alpha \leq 0.05$. Analysis of variance (ANOVA) was undertaken on all of the treatments within a given mine. Where a Treatment x Depth interaction was observed, a one-way ANOVA was undertaken with Treatment as the main effect and using parameters all from the same depth.

Results

Overview

Soil texture was similar between paired reclamation comparisons and undisturbed soils within each mine with the only exception being Mine 4 where the undisturbed soil was a sandy clay loam and the reclaimed soils were clays (Table 2). Soil EC and pH were also similar among compared practices as well as between reclaimed and undisturbed sites (Table 2). Undisturbed soils had pH and EC values that increased steadily with depth to 30 cm, while reclaimed soils had a more variable distribution. Bulk density of reclaimed soils ranged from 1.29 to 1.53 g cm^{-3} and for undisturbed soils from 1.27 to 1.38 g cm^{-3} (Table 2).

Weighted mean concentrations of SOC at undisturbed sites were always higher - with the exception of the grazing treatments at Mine 1 – than reclaimed sites (Table 2). There were, however, few clear patterns in regards to C:N ratios, and undisturbed soils were both higher and lower than reclaimed soils (Table 2).

Mean TN in reclaimed soils (0.585 mg N g $soil^{-1}$) was about 60% of undisturbed soils (0.962 mg N g^{-1} soil). In addition, the minimum value of TN in reclaimed soil was 0.190 mg N g soil compared to 0.458 mg N g $soil^{-1}$ found in undisturbed soil (Fig. 1). Results for MBN mirrored that of TN. Microbial biomass from undisturbed soil had a significantly greater (55%) amount of N than microbes from reclaimed soils (0.017 vs. 0.011 mg MBN g $soil^{-1}$). When the ratio of MBN:TN was compared, undisturbed soils (1.65) were 13% lower compared to reclaimed soils (1.87). Microbes in undisturbed soils appeared to take up N at a significantly ($P<0.1$) greater (35%) rate than microbes in reclaimed soils (Fig. 1).

Grazing

There were no significant differences between grazing treatments (grazed vs. un-grazed) at either mine. Undisturbed soils always had a greater concentration of TN (generally > 40%), but at Mine 1 the difference between undisturbed and un-grazed soils was statistically significant (Table 3) for the whole profile (0-30cm) as well as the 0-5, 5-15, 15-30 cm depth increments.

There was no difference in MBN between treatments or among undisturbed and grazing treatments at either mine (Table 3) except at the 0-5 cm depth increment at Mine 1, where both the grazed and un-grazed soils were significantly lower than the undisturbed soil.

Topsoil Handling

Topsoil handling treatments (direct haul vs. stockpiled) resulted in few differences in regards to TN but at both mines the two reclaimed treatments had significantly lower TN concentrations than undisturbed soils across most depth increments also (Table 3).

There was no difference in MBN between direct hauled and stockpiled soils at either mine. However, comparing handling treatments and undisturbed soils, there were different impacts on MBN. At Mine 1, directly hauled soils were significantly lower in MBN than undisturbed soil at the 0-5 and 0-30 cm depths. At Mine 3, stockpiled soil was significantly lower than the undisturbed soil at the 0-5 cm depth (Table 3).

Seed Mix

No differences in TN were observed in either mine due to the different seed mixes (grass vs. shrub). There were no differences in TN among either of the treatments and the undisturbed soil at Mine 4. At Mine 1 TN was significantly lower across all depth increments (Table 3).

Similar to topsoil handling treatments, MBN was highly variable across the different seed mixes used. At Mine 1, there was no difference between the two treatments across any soil depth. At Mine 4, MBN was significantly higher in the grass than the shrub treatment at the 0-5 and 0-30 cm depth increments. Between the undisturbed and seed mix treatments, MBN was significantly lower in shrub seeded soils at the 0-5 cm depth relative to the undisturbed soil in Mine 1, whereas at Mine 4, the grass seeded soils had a significantly lower MBN at the 0-5 cm depth increment (Table 3).

Mulches

Though the mean value for TN in stubble mulched soils was always greater than for hay mulched soils, the difference was not significant. Hay mulched soil was significantly lower in TN than the undisturbed soil at the 5-15, 15-30, and 0-30 cm increments though there was no difference between undisturbed and stubble mulched soil at any depth (Table 3).

Microbial biomass N paralleled TN with few differences apparent between the two mulch treatments. There were no differences between the undisturbed and stubble mulch treated soils at any depth except at the 5-15 cm depth increment but hay mulch soils were significantly lower in MBN at all soil depth increments except the 15-30 cm depth (Table 3).

Table 2. Site information and soil texture, clay, pH, electrical conductivity (EC), soil organic C (SOC) and C:N ratios in undisturbed and reclaimed soils for a series of surface coal mines located in southern and eastern Wyoming, USA. The data presented were calculated as a weighted mean of the 0-5, 5-15, and 15-30 cm depth increments.

Mine	Site	Reclamation Age (yrs)	Depth of replaced topsoil (cm)	Soil texture	Clay (%)	pH	EC (dSm^{-1})	SOC (g C kg^{-1} soil)	C:N[1]
Mine 1	Undisturbed	∞	-	Sandy clay loam	27	6.2	0.33	8.2	10.3
	Grazed[2]	26	30	Sandy clay loam	25	6.5	0.57	10.5	16.9
	Ungrazed	26	30	Sandy loam	16	6.3	0.58	10.2	21.3
	Direct Haul	11	45	Sandy clay loam	19	7.7	0.34	2.3	8.5
	Stockpile[3, 4]	11	45	Sandy clay loam	17	7.7	0.36	3.2	10.3
	Grass[3]	11	45	Sandy clay loam	17	7.7	0.36	3.2	10.3
	Shrub	11	45	Sandy clay loam	19	7.6	0.31	2.8	9.3
Mine 2	Undisturbed	∞	-	Sandy clay loam	18	6.2	0.34	13.5	15.7
	Grazed[5]	11	55	Sandy clay loam	18	7.2	0.41	5.8	12.3
	Ungrazed	11	55	Sandy clay loam	23	7.0	0.35	5.3	10.6
Mine 3	Undisturbed	∞	-	Sandy loam	17	8.0	0.63	13.1	13.1
	Direct Haul	19	35	Sandy loam	19	8.1	0.39	8.2	13.0
	Stockpile[6]	19	35	Loam	25	8.0	0.37	7.0	10.9
Mine 4	Undisturbed	∞	-	Sandy clay loam	34	6.6	0.42	9.5	11.0
	Grass	11	55	Clay	52	7.5	1.27	10.7	11.5
	Shrub	11	55	Clay	55	7.9	0.55	10.5	11.4
Mine 5	Undisturbed	∞	-	Sandy loam	20	7.8	0.42	8.5	10.6
	Hay	14	60	Sandy loam	16	8.1	0.36	6.7	14.6
	Stubble	14	60	Sandy loam	13	8.0	0.38	9.7	15.9

[1] Weighted total N data was taken from Table 3
[2] Grazed for *ca.* six years at a long duration (July – October) and low to moderate stocking rate
[3] These sites are the same, please refer to text for details
[4] Topsoil was stockpiled four years before reclamation was initiated
[5] Grazed for approximately five years at a short duration (two - 3 days periods) and high stocking rate (10 cattle ha^{-1})
[6] Topsoil was stockpiled five years before reclamation was initiated

Table 3. Means of total N (TN) and microbial biomass N (MBN) for a series of surface coal mines located in southern and eastern Wyoming, USA. The 0-30 cm increment was calculated as a weighted mean of 0-5, 5-15 and 15-30 cm depth increments. Means, by Mine, in columns, not followed by the same letter are statistically different at $P < 0.05$.

	Mine 1		Mine 2		Mine 3		Mine 4		Mine 5	
	TN	MBN	TN	MBN	TN	MBN	TN	MBN	TN	MBN
	g N kg^{-1} soil									
0-30 cm										
Undisturbed	0.80c	0.011b	0.86a	0.011a	1.00b	0.015a	0.86a	0.014ab	0.80b	0.011b
Grazed	0.62bc	0.012b	0.47a	0.010a	-	-	-	-	-	-
Ungrazed	0.48ab	0.006ab	0.47a	0.015a	-	-	-	-	-	-
Directhaul	0.27a	0.004a	-	-	0.63a	0.014a	-	-	-	-
*Stockpiled	0.31a	0.008ab	-	-	0.64a	0.009a	-	-	-	-
*Grass mix	0.31a	0.008ab	-	-	-	-	0.92a	0.008a	-	-
Shrub mix	0.30a	0.007ab	-	-	-	-	0.92a	0.018b	-	-
Hay	-	-	-	-	-	-	-	-	0.46a	0.005a
Stubble	-	-	-	-	-	-	-	-	0.61ab	0.007ab
0-5 cm										
Undisturbed	1.28c	0.031b	1.79a	0.027a	1.09a	0.043b	1.55a	0.045b	0.87a	0.024b
Grazed	0.90bc	0.012a	0.94a	0.026a	-	-	-	-	-	-
Ungrazed	0.77ab	0.009a	0.77a	0.021a	-	-	-	-	-	-
Directhaul	0.35a	0.008a	-	-	0.94a	0.031ab	-	-	-	-
*Stockpiled	0.49ab	0.019ab	-	-	0.90a	0.017a	-	-	-	-
*Grass mix	0.49ab	0.019ab	-	-	-	-	1.30a	0.014a	-	-
Shrub mix	0.39a	0.011a	-	-	-	-	1.34a	0.038b	-	-
Hay	-	-	-	-	-	-	-	-	0.54a	0.011a
Stubble	-	-	-	-	-	-	-	-	0.69a	0.013ab
5-15 cm										
Undisturbed	0.83c	0.009a	0.86b	0.009a	0.93a	0.014a	0.84a	0.008a	0.78b	0.012b
Grazed	0.66bc	0.011a	0.45a	0.007a	-	-	-	-	-	-
Ungrazed	0.56b	0.007a	0.45a	0.012a	-	-	-	-	-	-
Directhaul	0.27a	0.003a	-	-	0.68a	0.012a	-	-	-	-
*Stockpiled	0.29a	0.005a	-	-	0.64a	0.008a	-	-	-	-
*Grass mix	0.29a	0.005a	-	-	-	-	0.91a	0.007a	-	-
Shrub mix	0.27a	0.005a	-	-	-	-	0.89a	0.012a	-	-
Hay	-	-	-	-	-	-	-	-	0.45a	0.004a
Stubble	-	-	-	-	-	-	-	-	0.60ab	0.004a
15-30 cm										
Undisturbed	0.62b	0.005a	0.54a	0.006a	1.01b	0.006a	0.65a	0.008a	0.79b	0.006a
Grazed	0.50ab	0.012a	0.34a	0.005a	-	-	-	-	-	-
Ungrazed	0.33a	0.004a	0.45a	0.016a	-	-	-	-	-	-
Directhaul	0.25a	0.004a	-	-	0.49a	0.009a	-	-	-	-
*Stockpiled	0.28a	0.005a	-	-	0.56a	0.006a	-	-	-	-
*Grass mix	0.28a	0.005a	-	-	-	-	0.81a	0.007a	-	-
Shrub mix	0.29a	0.006a	-	-	-	-	0.80a	0.012a	-	-
Hay	-	-	-	-	-	-	-	-	0.45a	0.004a
Stubble	-	-	-	-	-	-	-	-	0.61ab	0.006a

*This site (comprised of stockpiled topsoil that had been seeded with a grass seed mix) is represented twice but compared separately against two different management comparisons; a site which had a grass mix seeded into direct haul topsoil site and a shrub seed mixture site which received stockpiled topsoil.

Table 4. Age of reclamation site, total N (TN) content, and ratio (relative to the undisturbed, native soil) of TN for a series of surface coal mines located in southern and eastern Wyoming, USA. Treatment means, for a given mine, in columns not followed by the same letter are statistically different at $P < 0.05$.

Mine	Site	Age (yrs)	TN[1] (kg N ha^{-1})	TN[2]	MBN[2]
Mine 1	Undisturbed	∞	3,111[c]	-	-
	Grazed	26	2,751[bc]	0.88	0.92
	Ungrazed	26	2,018[ab]	0.65	0.55
	Directhaul	11	1,143[a]	0.37	0.36
	Stockpile	11	1,327[a]	0.42	0.64
	Grass	11	1,327[a]	0.42	0.64
	Shrub	11	1,373[ba]	0.44	0.55
Mine 2	Undisturbed	∞	3,172[a]	-	-
	Grazed	11	2,084[b]	0.66	0.55
	Ungrazed	11	2,065[b]	0.65	1.45
Mine 3	Undisturbed	∞	3,816[a]	-	-
	Directhaul	19	2,579[a]	0.68	0.93
	Stockpile	19	2,649[a]	0.69	0.60
Mine 4	Undisturbed	∞	3,484[a]	-	-
	Grass	11	3,611[a]	1.04	0.57
	Shrub	11	3,648[a]	1.04	1.29
Mine 5	Undisturbed	∞	3,189[a]	-	-
	Hay	14	2,058[a]	0.65	0.45
	Stubble	14	2,606[a]	0.82	0.64

[1] Calculated as a weighted average based on depth increments (0-30 cm) and bulk density per depth

[2] Calculated as a weighted average and divided by the associated undisturbed soil value.

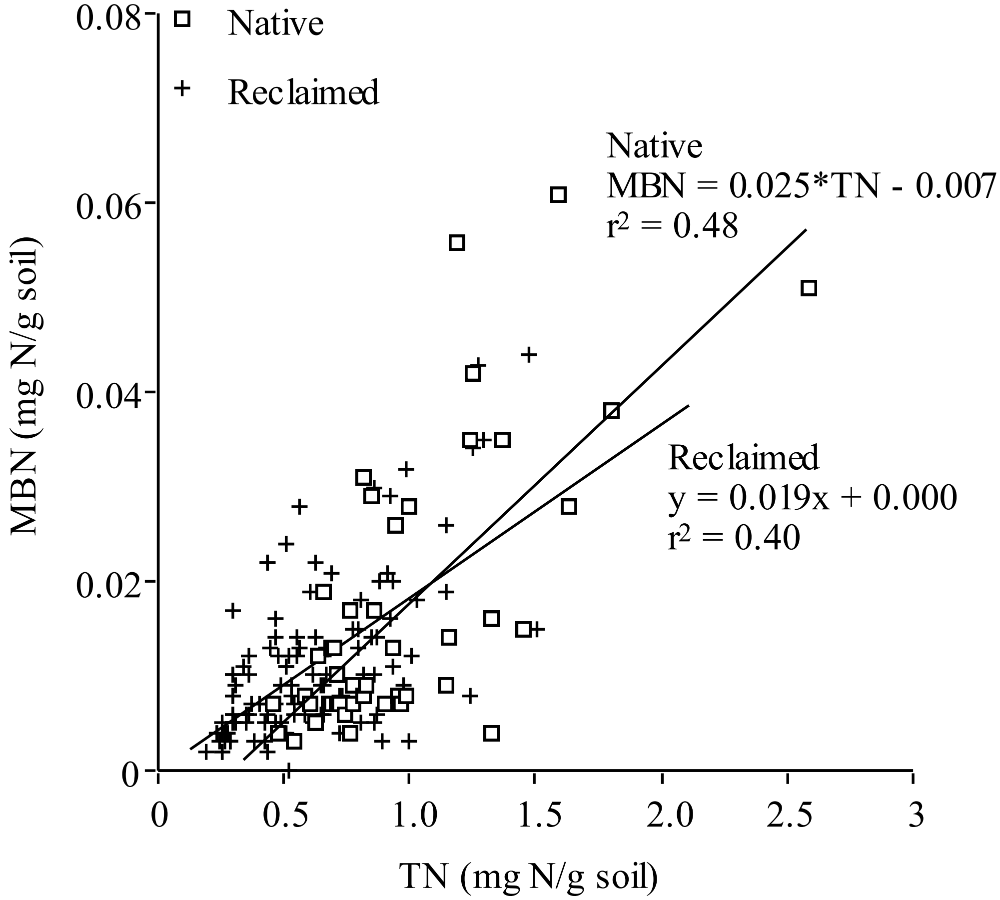

Figure 1. Total N (TN) plotted against microbial biomass N (MBN) in Native and Reclaimed soil from a number of coal mines in semi-arid Wyoming. There was a significant ($P = 0.0894$) difference in the gradient between reclaimed ($m = 0.187$) and native ($m = 0.253$) soils.

Discussion

From our results it is apparent that there was no consistent affect of any given management technique on soil TN content. While perhaps surprising, in previous work (Anderson et al., 2007) we found a similar situation in regards to SOC. In spite of this, it is still possible to make some observations about the potential effectiveness of management techniques on N accumulation.

Grazing appeared to have little influence on soil TN despite the two different patterns of grazing that we studied (moderate rates of grazing over a long time period, Mine 1 vs. high intensity over a very short period of grazing, Mine 2). While it is likely that heavy grazing will result in an appreciable loss of N out of the system as cattle graze forage, most of this N is returned back to the ecosystem in the form of excreta (approximately 85%; Heady and Child, 1994). It is also likely though that an unknown (but potentially significant) amount of N present in urine will be lost via volatilization.

While we would have expected to observe a greater mass of TN in direct hauled soils, relative to soil that had been stockpiled, we found no appreciable difference between these two treatments in this study. It is likely that this is the result of the amount of time (11-19 years) since the soils had been replaced and reclaimed and any differences present early on, had been negated by the 11+ years of growth and nutrient cycling of N.

Similarly for grass and shrub seed mixes there were negligible differences. While we might have expected that a grass community with its more fibrous root system would take up and accumulate more N, aboveground production was generally comparable between the two communities (L. Ingram, unpublished data, 2006). It is possible that over time, shrubs with their deeper roots (>2m; Sturges, 1977) will accumulate more soil N than a grass community.

Though not significant, the much greater accumulation of TN (27%; Table 4) may indicate that a stubble crop is of value in that it may be taking N up from deeper in the profile but may also assist in protecting soil from erosion to a greater extent than the use of hay mulch alone.

We are unsure why soils at Mine 4 that had been reclaimed with grass and shrubs had a mass of TN that exceeded (though not significantly) that of the undisturbed soil after a relatively short period of time (11 yrs). Though this site had a greater mean annual precipitation than the other sites (17+ %) and there was significant correlation between TN concentration and mean annual precipitation (P=0.0024, r^2=0.072, n=126), with such a low estimate of variance it is unlikely that precipitation is the major factor influencing the accumulation of soil N.

Results for MBN also suggest that microbial communities are quite different in undisturbed vs. reclaimed ecosystems. We observed that though microbes from undisturbed soils contained less N than microbes from reclaimed communities, they took up N at a greater rate. This would seem to support a hypothesis that we put forward previously (Ingram et al., 2005) in which we suggested that there is a greater supply of labile material present in reclaimed soils despite lower overall concentrations of TN. In contrast, undisturbed soils have a greater mass of TN, but much of it is present in more humified soil organic matter (SOM) fractions, and this N is less available for microbial uptake. Micro-organisms from reclaimed soils, however, appear to be better adapted to taking up N under low N concentrations (approximately 1%; Fig. 1). This probably occurs because development of a microbial community parallels that of a vegetation community: an early initial successional microbial population over time becomes more diverse and specialized over time as it develops into a later successional population (Frequez and Aldon, 1984).

Nitrogen is almost always inherently low in arid and semiarid ecosystems (Charley and Cowling, 1968) and Wyoming soils are no different in this regard. With the exception of Mine 4, reclaimed soils had a concentration/mass of soil N that was approximately 40% lower than undisturbed soil up to 26 yrs after being reclaimed. That these soils are much lower in TN is not at all surprising. Total N in most soils is strongly correlated with SOC/SOM and 30-85% SOM can be lost due to stockpiling (Abdul-Kareem and McRae, 1984). Though C recovers more quickly than N (approximately 13% faster, relative to N, on the same sites and time since reclamation; Anderson et al., 2007), it is likely that N is also not lost to the same extent that C is during the course of mining and reclamation (i.e., through the processes of soil removal, storage, relaying, regrading, and seeding). This is probably due to the fact that whereas C is mineralized as an energy source and is subsequently lost as CO_2, N becomes incorporated into microbial biomass and is protected from loss. Across the undisturbed native sites (Table 4) there was remarkably little variation among all five sites (mean = 3,354 kg N ha^{-1}, SD = 296, Coefficient of Variation = 9%). This suggests soils present in these ecosystems appear to have a maximum capacity to store N and there is no reason to believe that soils on which reclamation treatments were carried out would be any different.

Having noted that N can be lost in a number of ways as a result of mining and reclamation it is of interest to ask the question, how does N accumulate in a reclaimed soil? There are a number of potential ways in which this may occur: 1) The addition of fertilizer; 2) N-fixation by leguminous plant species; 3) N-fixation by free-living micro-organisms; 4) Associative N fixation 5) Wet/dry deposition.

Fertilizer is rarely used as an integral part of most reclamation in Wyoming coal mines.

Nitrogen-fixing plants, such as legumes, are best known for the symbiotic partnership they form with *Rhizobia* and *Bradyrhizobium* bacteria allowing them to fix N. As a result of the high energy costs required for N to be fixed (Marschner, 1995) and that fixation may be controlled by other limiting factors (P, Mo; Schlesinger, 1997), leguminous plants are often facultative rather than obligate. Leguminous species, though present in many of the seed mixtures used in reclamation, tend to be only a very small component of species represented (A. Wick, pers. comm.).

Associative N fixers (e.g., *Azosporillum*, *Azotobacter*) are organisms found around plant roots (rhizosphere association) that use root exudates as their primary C source. While the amount of N they fix is often greater than that fixed by free-living N fixers, much of this N only becomes available after the micro-organism dies (Marschner, 1995, Sylvia et al., 1999). Because associative fixers rely on root exudates for their C source they are likely to be nutrient and energy limited in arid and semiarid ecosystems. As a general rule for a range of grassland ecosystems it has been estimated that between 5 to 25 kg N ha^{-1} is fixed annually (Sylvia et al., 1999).

Free-living heterotrophic (require a C source for energy) bacteria are also able to fix N. Because of their reliance on external sources of C to provide energy their ability to fix N is often limited. For instance it has been suggested that due to C limitations, free-living microbes fix less than 1 kg N ha^{-1} yr^{-1} (Bothe et al., 1983 cited in Marschner, 1995). Other free-living micro-organisms are autotrophic (produce their own C through photosynthesis) and are less limited in their ability to fix N. Probably the best known of the autotrophic microbes are cyanobacteria (also know as blue-green algae) and it has been estimated that they can fix 25 kg N ha^{-1} $yr\text{-}^{1}$ (Sylvia et al., 1999)

It is very difficult however to estimate the amount of N fixed by microbes. Numerous studies have estimated that these various microbes could theoretically fix up to several hundred kilograms of N per hectare per year, though it is likely that in reality they fix much less due to climatic, soil water, and substrate constraints. In addition, it should be noted that the methods by which N-fixation is estimated (whether by symbiotic relationships, free-living or associative relationships) all have their limitations and we are not able to obtain truly accurate measures of the amount of N-fixed (and that ends up in soil).

Wet and/or dry deposition probably provides only small quantities of N to these reclaimed ecosystems. It is estimated that in the order of 2 kg N ha^{-1} yr^{-1} (wet and dry) is deposited in the Powder River Basin (Holland et al., 2005) though in some comparable ecosystems (Pawnee National Grasslands, CO, and the Cottonwood Range and Livestock Research Station, SD) it is estimated that depositions of 6-9 kg N ha^{-1} yr^{-1} (wet and dry) have been recorded (Woodmansee, 1978). This estimate probably underestimates actual N deposition as it does not include entities such as NO_2 and NO_x gas (as a result of blasting) and which may be significant sources of dry deposition.

The results of our current work though give rise to somewhat of a conundrum, in that TN is almost always lower in reclaimed soils (approximately 30% lower) but most of the time plant productivity is greater (74% of reclaimed sites averaged 77% greater production relative to undisturbed sites; Ingram et al., 2005 and L. Ingram, unpublished data, 2006). The reasons for this are not entirely clear. In addition there was no relationship between production/mass of N present in vegetation and soil TN (L. Ingram, unpublished data, 2006) which leads us to conclude N does not appear to be limiting production in these reclaimed ecosystems. While TN tells us little about the availability of inorganic N (NO_3 + NH_4 – both of which are required for plant growth) it is a reasonable expectation that an increase in N-mineralization will result in greater plant growth. We have previously observed a strong relationship between TN and N-mineralization in reclaimed soils (P = 0.0001, r^2 = 0.70, n = 275; L. Ingram, unpublished data) and thus in soils which average $^1/_3$ less TN, it seems unlikely that N-mineralization would be greater in reclaimed soils. In addition, though N-mineralization is often greater in reclaimed relative to undisturbed soils, it is not an across the board phenomena (Ingram et al., 2005).

Thus we are left with no obvious reason as to why production is consistently greater on reclaimed sites (relative to undisturbed native sites) despite containing lower amounts of TN though we speculate that a number of reasons that may contribute to it. A number of studies have noted that soil stored in stockpiles tends to accumulate NH_4 at depth (Ross and Cairns, 1981; Williamson and Johnson, 1990) and this by acting as a fertilizer could initially give rise to greater growth. While this explains some of the observed patterns of growth, it does not explain why soils that were directly hauled or soil that has been reclaimed for decades (in some cases) also had greater production. Part of this explanation may have to do with the species planted into reclaimed soils are more productive under these low soil fertility conditions, however, as the species planted are typically native species this appears to be an unlikely reason. It is more probable that in undisturbed, northern mixed-grass ecosystems (Coupland, 1992), there is a more equal distribution of cool-season and warm-season grass species. In a reclaimed situation, though warm-season grasses are often seeded into reclaimed sites they are often out competed by cool-season grasses, and cool-season grasses are typically more productive than warm-season grasses (Dijkstra et al., 2006). Finally, reclaimed sites typically have a greater plant density than found on undisturbed, native sites, which leads to greater productivity (G. Schuman, pers. comm.).

Nitrogen though low in most reclaimed soils, does not appear to limit production. Our results seem to suggest that no given management practice gives rise to greater soil N over any other. There appears to be little that can be done to improve TN directly as the only option available for most reclamation specialists to improve TN is to fertilize but high costs and the greater likelihood of weed species spreading, means fertilization is not an option in most cases. However, in light of the generally greater vegetation production that occurs in reclaimed sites over a relatively short time frame (10^+ yrs), low concentrations of N does not appear to be limiting production. In addition an increased proportion of cool-season grasses present in the seeded native communities is probably a major contributing factor to the greater aboveground production observed. Over time natural process will result in a gradual, though slow, increase of TN.

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Literature Cited

Abdul-Kareem, A.W. and S.G. McRae. 1984. The effects on topsoil of long-term storage in stockpiles. Plant Soil 76: 357-363. http://dx.doi.org/10.1007/BF02205593.

Anderson, J.D., L.J. Ingram, P.D. Stahl, 2007. Influence of Reclamation Management Practices on Microbial Biomass Carbon and Soil Organic Carbon in Semiarid Mined Lands of Wyoming. Appl. Soil Ecol. (In review).

Blake, G.R., Hartge, K.H. 1986. Bulk Density. p. 363-375. *In* A. Klute (ed.) Methods of Soil Analysis, Part 1, Physical and Mineralogical Methods. 2nd edition, SSSA Book Series No. 5. Agronomy Society of America, Madison, WI.

Bothe, H., M.G. Yates, and F.C. Cannon. 1983. Physiology, biochemistry and genetic dinitrogen fixation. p 241-285. In A. Läuchli and R.L. Bieski (eds) Encyclopedia of Plant Physiology, New Series. Vol 15A. Springer-Verlag, Berlin and New York. http://dx.doi.org/10.1007/978-3-642-68885-0_9.

Cabrera, M.L. and M.H. Beare. 1993. Alkaline persulfate oxidation for determining total nitrogen in microbial biomass extracts. Soil Science Society of America Journal 57; 1007-1012. http://dx.doi.org/10.2136/sssaj1993.03615995005700040021x.

Charley, J.L., and S.W. Cowling. 1968. Changes in soil nutrient status resulting from overgrazing and their consequences in plant communities of semi-arid areas. Proc. Ecol. Soc. Aust. 3: 23-38.

Coupland, J.T. 1992. Mixed Prairie. In R.T Coupland (ed.) Ecosystems of the World. 8A. Natural Grasslands Introduction and Western Hemisphere. Elsevier, Amsterdam and New York.

Dijkstra, F.A., S.E. Hobbie, and P.B. Reich. 2006. Soil processes affected by sixteen grassland species grown under different environmental conditions. Soil Sci. Soc. Amer. J. 70: 770-777. http://dx.doi.org/10.2136/sssaj2005.0088.

Forster, J.C. 1995. Determination of Water Holding Capacity of Soils. p. 106. *In* K. Alef and P. Nannipeiri (eds). Methods in Applied Soil Microbiology and Biochemistry. Academic Press, San Diego, CA.

Fresquez, P.R. and E.F. Aldon. 1984. Distribution of fungal genera in stockpiled topsoil and coal mined spoil overburden. USDA Forest Service Resarch Note RM-477. Rocky Mountain Forest and Range Experiment Station, Fort Collins, CO.

Gee, G.W. and D. Or. 2002. Particle-Size Analysis. p. 255-316. *In* J.H. Dane and G.C. Topp (eds) Methods of Soil Analysis, Part 4, Physical Methods. SSSA Book Series 5. Agronomy Society of America, Madison, WI.

Heady, H.F. and R.D. Child. 1994. Rangeland Ecology and Management. Westview Press, Boulder.

Holland, E. A., B.H. Braswell, J. Sulxman, and J.-F. Lamarque. 2005. Nitrogen deposition onto the United States and Western Europe: Synthesis of observations and models. Ecol. Applic. 15: 38-57. https://doi.org/10.1890/03-5162

Koss, W.J., J.R Owenby, P.M. Steurer, S.E. Devoyd. 1988. Freeze/Frost data. Climatography of the U.S., No. 20, Supplement No. 1. National Climatic Data Center. Ashville, North Carolina. [online] Available at http://ncdc.noaa.gov/oa/climate/freezefrost/freezefrost.pdf (verified 19 Sept.. 2006).

Ingram, L.J., Schuman, G.E., Stahl, P.D., Spackman, L.K., 2005. Microbial respiration and organic C indicate nutrient cycling recovery in reclaimed soils. Soil Sci Soc. Am. J. 69, 1737-1745. http://dx.doi.org/10.2136/sssaj2004.0371.

Jenny, H., 1930. A study on the influence of climate upon nitrogen and organic matter content of the soil. Research Bulletin No. 152. University of Missouri.

Marschner, H. 1995. Mineral Nutrition of Higher Plants. Second Edition. Academic Press. London and San Diego.

Munn, L.C. and C. S. Arneson. 1999.1:100,000-Scale Digital Soils Map of Wyoming: University of Wyoming Agricultural Experiment Station. [Online]. Available at http://www.wygisc.uwyo.edu/24k/soil100.html (Verified 19 Sep. 2006).

Paul, E.A. and F.E. Clark. 1996. Soil Microbiology and Biochemistry. Second Edition. Academic Press, San Diego and London.

Rhoades, J.D. 1996. Salinity: Electrical Conductivity and Total Dissolved Solids. p. 417-435. *In* D.L. Sparks (ed.) Methods of Soil Analysis, Part 3, Chemical Methods. SSSA Book Series No.5 Soil Science Society America and American Society of Agronomy. Madison, WI.

Ross, D.J. and A. Cairns. 1981. Nitrogen availability and microbial biomass in stockpiled soils in Southland. N.Z. J. Sci. 24:137-143.

Schlesinger, W.H.1997. Biogeochemistry. An Analysis of Global Change. Second Edition. Academic Press. San Diego and London.

Sherrod, L.A., Dunn, G., Peterson, G.A., Kolberg, R.L., 2002. Inorganic carbon analysis by modified pressure-calcimeter method. Soil Sci. Soc. Am. J. 66, 200-305. http://dx.doi.org/10.2136/sssaj2002.0299.

Sturges, D.L. 1977. Soil water withdrawal and root characteristics of big sagebrush. Am. Midl. Nat. 98: 257-273. http://dx.doi.org/10.2307/2424978.

Sylvia, D.M., J.J. Fuhrmann, P.G. Hartel, and D.A. Zuberer. 1999. Principles and Applications of Soil Microbiology. Prentice Hall, New Jersey.

Thomas, G.W. 1996. Soil pH and Soil Acidity. p. 475-490. *In* D.L. Sparks (eds) Methods of Soil Analysis, Part 3, Chemical Methods. SSSA Book Series No.5, Soil Science Society of America and American Society of Agronomy. Madison, WI.

Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. Soil Biol. Biochem. 19, 703-707. http://dx.doi.org/10.1016/0038-0717(87)90052-6.

Westerman J.W., Prink, C., 2004. Soil Survey of Campbell County, Wyoming, Southern Part. Natural Resource Conservation Service. U. S. Department of Agriculture. Washington.

Western Region Climate Center. 2004. Wyoming Climate Summaries. [Online]. Available at http://www.wrcc.dri.edu/summary/climsmwy.html (verified 18 Sep. 2006).

Williamson, J.C. and D.B. Johnson. 1990. Mineralisation of organic matter in topsoils subjected to stockpiling and restoration at opencast coal sites. Plant Soil 128:241-247. http://dx.doi.org/10.1007/BF00011115.

Woodmansee, R.G. 1978. Additions ands losses of nitrogen in grassland ecosystems. BioScience 28: 448-453. http://dx.doi.org/10.2307/1307227