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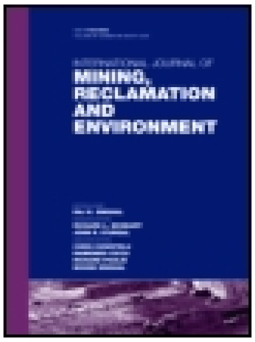

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Some physiological characteristics to estimate species potential as a mine reclamation ground cover

Eddy Nurtjahya[a] and Jennifer A. Franklin[b]

[a]Department of Biology, Universitas Bangka Belitung, Bangka Belitung, Indonesia; [b]Department of Forestry, Wildlife and Fisheries, University of Tennessee, Knoxville, TN, USA

ABSTRACT
In finding what physiological characteristics can be used to predict ground cover success on mine reclamation sites, 14 herb and grass species were tested. Establishment and early growth was tested on three different soils, ie vermiculate and quartz sand mixture, quarry overburden and coal mine overburden in a greenhouse. The results indicate that plant height and cover, transpiration rate and foliar pigments may be used to select plant adaptability to mined soil. White clover (*Trifolium repens* L.) showed the greatest potential as ground cover for mined soils. Species most widely used in reclamation tended to be perennials of moderate rate.

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Physiological characters; cover crops; reclamation; mine revegetation; white clover

Introduction

The planting of vegetation to stabilise soil disturbed by mining has become common practice in much of the world, and is a requirement in many countries. A primary goal of revegetation, upon which many regulations are based, is to stabilise surface materials reducing the movement of sediment into surface waters and prevent the development of erosional landforms. The visual improvement of mined landscapes is another common goal, prompting revegetation activities on both newly mined land and older mine spoils where vegetation has not developed spontaneously. The establishment of vegetation by direct seeding is an economically attractive option and the selection of herbaceous species is often based on seed cost and availability, and on the reliability of vegetation establishment. Regional ground cover mixtures are often developed by trial and error. Agronomic species are commonly used because seeds are readily available, relatively low in cost, planting dates and methods are well established, and in many cases are available as widely adapted cultivars. Seeding trials have identified species or cultivars tolerant of adverse chemistry, such as high metal concentrations of some mine spoils [1]. Identification and demonstrated success of some species for mine reclamation has led to their widespread use on regional and even global scales over the past few decades.

Land rehabilitation, activities that lead to an improvement in land function, differs from ecological restoration in that the goal of the latter is to create an ecosystem that is diverse and resilient, containing locally adapted species assemblages [2]. In reclaiming mined lands, rehabilitation is now readily achievable in many cases and an ecosystem restoration is now recognised as being a preferable goal. On former coal mines in the eastern US, the restoration of native vegetation has resulted in the restoration of some ecosystem services at levels similar to the pre-mined condition [3]. In Europe mined lands have been recognised for their biodiversity and as refugia for endangered species [4].

CONTACT Eddy Nurtjahya eddy_nurtjahya@yahoo.com, ebudihar@utk.edu

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While it is clear that the use of native species is preferable, species must be regionally selected based on local species assemblages and site characteristics of the reclaimed mine. There is an additional challenge in cases where reclaimed soils are contaminated with metals, are highly acidic or alkaline, or coarsely textured. There has been much recent interest in the use of functional or ecophysiological traits as a tool for the selecting appropriate species for specialised situations, but few studies have investigated this approach in the context of mine reclamation. The evaluation of 10 native tree species in tin mined soil in Bangka Belitung islands concluded that 4 of 10 species showed the highest survival rates and cover development [5] but measurement of stomatal density, epidermal cell thickness, cuticle thickness, palisade and spongy mesophyll thickness, root conductivity and root conductivity ratio, chlorophyll and nitrogen contents of five year saplings of those species, best adapted species were not those that showed the best performance in the field [6]. The identification of traits that can be used to identify species for potential use in mine reclamation would greatly speed the search for appropriate native species.

The goal of this study is to identify some characteristics of early growth and establishment that could be used for local rapid screening to identify promising native mine reclamation species. We hypothesise that species whose use has been widely adopted are those that are able to germinate and establish under a wide range of soil conditions. We tested germination rates in the over a wide pH range, and the early growth, pigment content and transpiration rates of 14 herbaceous species to determine whether these traits might predict ground cover success on mine reclamation sites, and assess whether a trait-based approach for the selection of plant species may be feasible for mine reclamation.

Methods

Plant species and growth conditions

The experiment was carried out with 14 herbs and grass species [7] (Table 1) with selection based on availability, functional groups and use in mine reclamation. All species have seeds are widely available commercially within the south-eastern United States at a reasonable cost, and widespread use in regional agriculture, suggesting that they could be readily adopted by the mining industry. Our selection was made to include the functional groups of grasses (6 species), legumes (4 species) and other herbaceous species (4). These species were also selected to include both annuals (6 species) and perennials (8 species). We were interested in including species that are widely used in reclamation as well as those that are grown agriculturally but rarely used in reclamation. A literature search was conducted using several agricultural databases for references to planted groundcover, and publications

Table 1. List of plant species.

Species	Common name	Family	Life history	# Refs
Brassica napus L.	'Dwarf essex' rape	Brassicaceae	Annual	39
Brassica perviridis (L.H. Bailey) L.H. Bailey	'Tendergreen' mustard	Brassicaceae	Annual	0
Dactylis glomerata L.	*Orchard grass	Poaceae	Perennial	50
Hibiscus esculentus L.	Okra 'red burgundy'	Malvaceae	Annual	2
Lolium multiflorum Lam.	*Annual ryegrass	Poaceae	Annual	20
Lolium perenne L.	*Perennial ryegrass	Poaceae	Perennial	29
Lotus corniculatus L.	*Birdsfoot trefoil	Fabaceae	Perennial	30
Panicum virgatum L.	Switch grass 'Alamo'	Poaceae	Perennial	38
Polygonum fagopyrum L.	Buck wheat	Polygonaceae	Annual	1
Schizachyrium scoparium (Michx.) Nash	Little bluestem	Poaceae	Perennial	34
Sorghastrum nutans (L.) Nash	Indian grass	Poaceae	Perennial	43
Trifolium pratense L.	Red clover	Fabaceae	Perennial	21
Trifolium repens L.	*Ladino clover	Fabaceae	Perennial	23
Vigna unguiculata L.(Walp.)	Cow pea 'Texas C40'	Fabaceae	Annual	0

Notes: Species used in the experiment, and the frequency with which these are referenced in the literature as being present on, or seeded onto, reclaimed mine sites.
*Currently in widespread use in surface mine reclamation in the eastern US.

specialising in mine reclamation were searched back to 1980, and the number of citations counted for each species. Of the 14 species tested here, 4 are recommended for mine reclamation and have been widely used [8], 6 have received moderate use either historically or regionally, and 4 have little or no record of use although they are widely planted for other uses within the region.

Annual rye (*Lolium multiflorum*), perennial ryegrass (*Lolium perenne*) and ladino clover (*Trifolium repens*) are currently recommended as tree compatible ground covers for reclamation of eastern coal mines with and end land use goal of forestry [8]. Annual rye is a widely distributed introduced grass that is used to provide rapid vegetative cover, but does not persist past the first growing season. Birdsfoot trefoil (*Lotus corniculatus*) and orchardgrass (*Dactylis glomerata*) are recommended additional species for steep slopes. As an alternative to these non-native pasture species, Indian grass (*Sorghastrum nutans*) and little bluestem (*Schizachyrium scoparium*) are native warm season grasses that are also recommended. These native grasses are widely distributed in the US east of the Rocky Mountains, grow in upright bunches, and are adapted to drought and to a wide range of soil conditions. Switchgrass (*Panicum virgatum*) has been widely tested in recent years as a potential biofuel crop for reclaimed lands. Red clover (*Trifolium pratense*) was historically widely used in reclamation, but is now used less often in favour of the recommended legumes listed above. The remaining five species are little used in reclamation although they are common agricultural annuals in the region, and tolerant of nutrient-poor soils. All seeds were obtained from a commercial supplier.

Seed germination

The pH of mine spoils can be widely variable, and while spoils below a pH of 5 are often treated to increase pH, the treatment of high pH spoils is more difficult and revegetation may rely on the selection of tolerant plant species. To test the influence of pH on germination, seeds were germinated on Whatman filter paper in 9-cm sterile plastic Petri dishes, using 50 seeds per dish. The paper was moistened with 3–6 ml of solution at pH 5, 6, 7, 8, 9, 10 or deionised water as a control. Because the upper pH limits for germination are not known for these species, a pH up to 10 was used to ensure the identification of the upper limit of each species range. A 2 mM solution of MES [2–(*N*–morpholino) ethanesulphonic acid] was adjusted to pH 5 or 6 with 1 N NaOH. A 2 mM solution of HEPES [N–2–hydroxymethyl) piperazine–*N′* – (2–ethanesulphonic acid)] was adjusted to pH 7 or 8 with 1 N NaOH. A pH 9 or 10 buffer was prepared with 2 mM tricine [N–tris(hydroxymethyl) methylglycine] and adjusted with 1 N NaOH. Petri dishes were sealed with parafilm and placed in a growth chamber with a 12-h temperature cycle of 25 °C/35 °C, a 24-h photoperiod with lighting provided by cool white fluorescent bulbs. Germination was determined by the emergence of radicle, and was assessed every 2–3 days. At each assessment period seeds with radicles emerged were counted and removed and the dish was sealed and returned to the chamber until no further germination was recorded between one assessment period and the next. This test assessed only germination, and establishment was assessed in the greenhouse tests. The experimental design was completely randomised with one factor (pH) and was composed of three replicate dishes for each species and pH treatment.

Establishment and early growth

Seeds were sown in PVC pots (305 mm height and 100 mm diameter) on three different soil types: a vermiculite and quartz sand mixture at 1:1 (v:v) ratio, quarry overburden and coal mine overburden (Table 2). High seeding rates, with seeds nearly covering the soil surface, were used to ensure an adequate number of germinants to theoretically provide 100% ground cover even in species with low germination rates. The quarry overburden was a weathered, unconsolidated, stony, silt and clay overlying a limestone bedrock collected from a quarry in Oak Ridge, Tennessee. Coal overburden was a sediment produced by the rapid breakdown of a mixture of shale and weathered and unweathered -sandstones, and was collected from a newly reclaimed coal mine near Elk Valley, Tennessee. Each treatment was repeated three times as blocks. The plants were grown in the University of Tennessee – Knoxville greenhouse

Table 2. Substrate properties.

Media	Location	Texture	Age since extraction	Vegetative condition	pH H_2O	P	K	Ca	Mg	Zn	Cu	Fe	Mn	Na	Organic matter	Bulk density
						kg ha^{-1}									%	
Vermi/Sand		–	–		9.5	6.7	190.9	533.5	125.5	3.1	5.5	16.1	2.2	1556.9	0.1	–
Quarry OB	Oak Ridge, Tennessee	Clay loam	2 yrs	Bare	6.1	0.4	54.2	3626.0	1854.3	17.3	0.4	3.7	29.5	18.7	0.7	1.93
Coal OB	Elk Valley, Tennessee	Sandy loam	3 yrs	Ground cover	7.6	263.0	180.5	3795.2	1311.4	21.8	8.0	32.5	229.8	72.9	2.2	1.81

Note: Chemical properties of vermiculite and sand mixture at 1÷1 (v÷v) ratio, quarry overburden, and coal mine overburden.

under natural lighting with three 1000-watt mercury lamps to extend the photoperiod to 15 h, and 23.9–27.7 °C/18.3–21.1 °C (day/night) temperature and 48.0/48.5% (day/night) average humidity during the study period. The plants were watered with tap water in the morning and afternoon daily during the study.

Plant growth measurements

Plant height and cover were measured at weeks 2, 4, 6 and 8 after seeding. For plant height, three randomly selected individual seedlings were measured in each pot. Plant cover was estimated visually from directly above the pot.

Transpiration measurement

Transpiration rate, using a Li–1600 chamber steady-state porometer (Li–COR, USA), was measured at 10:00 – 14:00 beginning 11 weeks after planting. Measurements were made over nine consecutive days. Leaf area was measured using a Li–3100 Area Metre (Li–COR, USA). Shoots were harvested and stored at −4 °C for later pigment analysis.

Leaf pigment concentrations

To obtain enough tissue for analysis, shoot tissue was pooled for each species and soil type. Approximately 0.2 g of leaf tissue was extracted in 80% (v÷v) acetone. Pigments in extracts were determined colorimetrically (Bio-Mate 3, Thermo Scientific, USA) at wavelengths of 480, 663 and 645 nm, and foliar concentrations calculated based on fresh weight using equation [9] for chlorophylls a and b, and equation [10] for carotenoids.

Statistical analyses

Test for outliers and normality was carried out; all values which were out of biological range were removed, and variables that did not meet the assumptions of normality were log transformed prior to analysis. A general linear model was used to test for main effects of species and soil type, and week where applicable. Where a significant interaction between species and soil type was found, species were tested separately to determine whether soil effects were significant, using a Tukey post hoc to test for differences between means. All analysis was completed using SPSS © Statistical Software (version 18.1, SPSS Inc., Chicago, USA).

Results

Average germination ranged from 2% to 49% (Table 3), with commonly used species having both high and low germination percentages over the test period. Germination was sensitive to pH in only two species, *B. napus* and *V. unguiculata*, which are rarely used in reclamation. *B. napus* germination was the greatest at pH 5 and 6 (49%), then decreased to a low at pH 10 (40%). *V. unguiculata* had its greatest germination at pH 7 and 9 (26% and 33%) and lowest at pH 5 and 10 (11%).

Establishment and early growth was more sensitive to soil chemistry. When measured at eight weeks after seeding, mean plant cover differed significantly between species and between soil types ($p < 0.001$). Across all species, cover was greatest in quarry overburden (66%), least in the vermiculite and quartz sand mixture (49%), and intermediate in the coal overburden (58%). Eight species, *T. pratense*, *T. repens*, *B. napus*, *B. perviridis*, *P. fagopyrum*, *L. multiflorum*, *V. unguiculata* and *H. esculentus* rapidly developed shoots which provided more than 50% ground cover within two weeks of planting (Table 3), while other species had a much slower rate of development. Three grass species, *L. perenne*, *S. scoparium* and *S. nutans*, did not attain 50% coverage over the eight week study period.

Eight species showed a significant interaction between soil type and cover development (Figure 1). Of these, all had less foliar cover in the sand mixture with the exception of *L. multiflorum*, which had less cover in the coal overburden. *L. multiflorum* had the most rapid height growth of all tested species (Figure 2), with height growth being insensitive to soil type (Figure 3). Height growth was sensitive to soil type in all of the other tested grass (monocot) species, being lower in the sand mixture, and all showed steady height growth throughout the study period. Three dicot species, *P. fagopyrum*, *H. esculentus* and *V. unguiculata*, showed early and steady height over the study period, while five showed slow growth that appeared to stall after four weeks (Figure 2). *T. pratense* and *L. corniculatus* were the only dicot species tested whose height growth performed consistently well across all three soil types.

Table 3. Germination and early growth. Average (± standard error) germination and early growth percentages of 14 species tested over a pH range from 5 to 10, and early growth and establishment averaged across 3 soil types.

	Germination (%)	Cover at two weeks (%)	Cover at eight weeks (%)
B. napus	44 ± 1.2	93 ± 9.0	73 ± 6.2
B. perviridis	48 ± 0.9	84 ± 7.3	69 ± 4.6
D. glomerata	16 ± 2.0	36 ± 6.5	52 ± 7.8
H. esculentus	49 ± 0.3	55 ± 10.2	51 ± 7.9
L. multiflorum	10 ± 0.7	50 ± 5.5	50 ± 4.3
L. perenne	46 ± 2.3	26 ± 4.4	35 ± 4.4
L. corniculatus	12 ± 1.0	43 ± 7.3	71 ± 9.6
P. virgatum	21 ± 0.8	26 ± 4.0	38 ± 5.5
P. fagopyrum	2 ± 0.3	66 ± 10.6	68 ± 7.6
S. scoparium	5 ± 0.5	10 ± 2.0	10 ± 2.7
S. nutans	35 ± 1.2	15 ± 3.0	13 ± 2.7
T. pratense	46 ± 0.6	100 ± 3.3	92 ± 5.5
T. repens	37 ± 1.5	62 ± 7.8	78 ± 8.9
V. unguiculata	16 ± 2.8	59 ± 12.9	78 ± 5.2

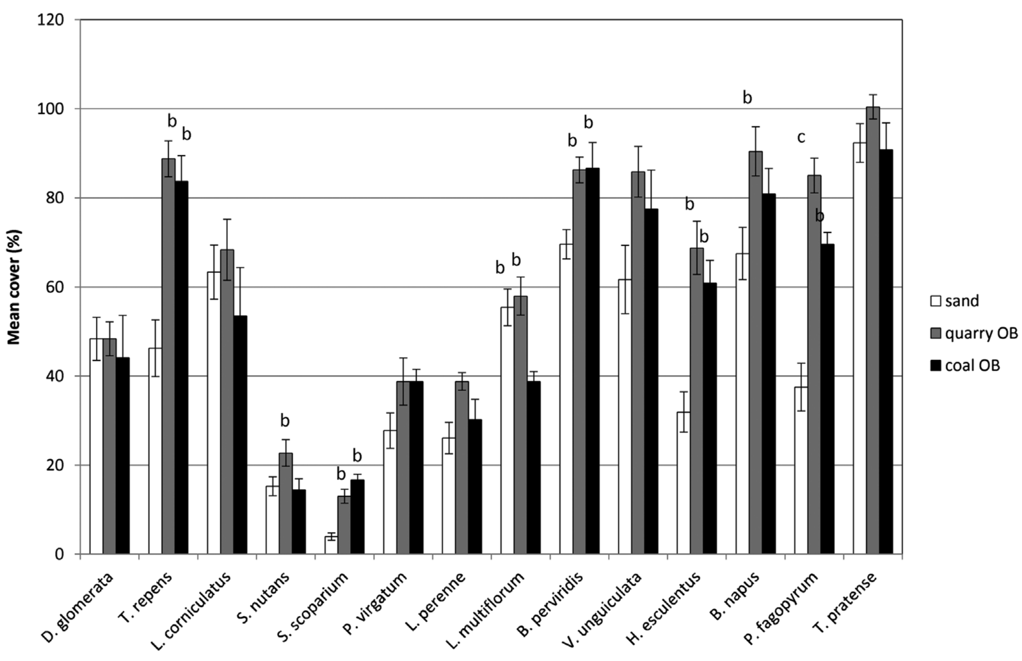

Figure 1. Foliar cover. Mean percentage of foliar cover in three different soil types (vermiculate and quartz sand mixture at 1÷1 (v÷v) ratio, quarry overburden, and coal mine overburden), averaged over the eight week study period. Bars indicate standard error. Different letters indicate differences between soil types for a given species at $p < 0.05$.

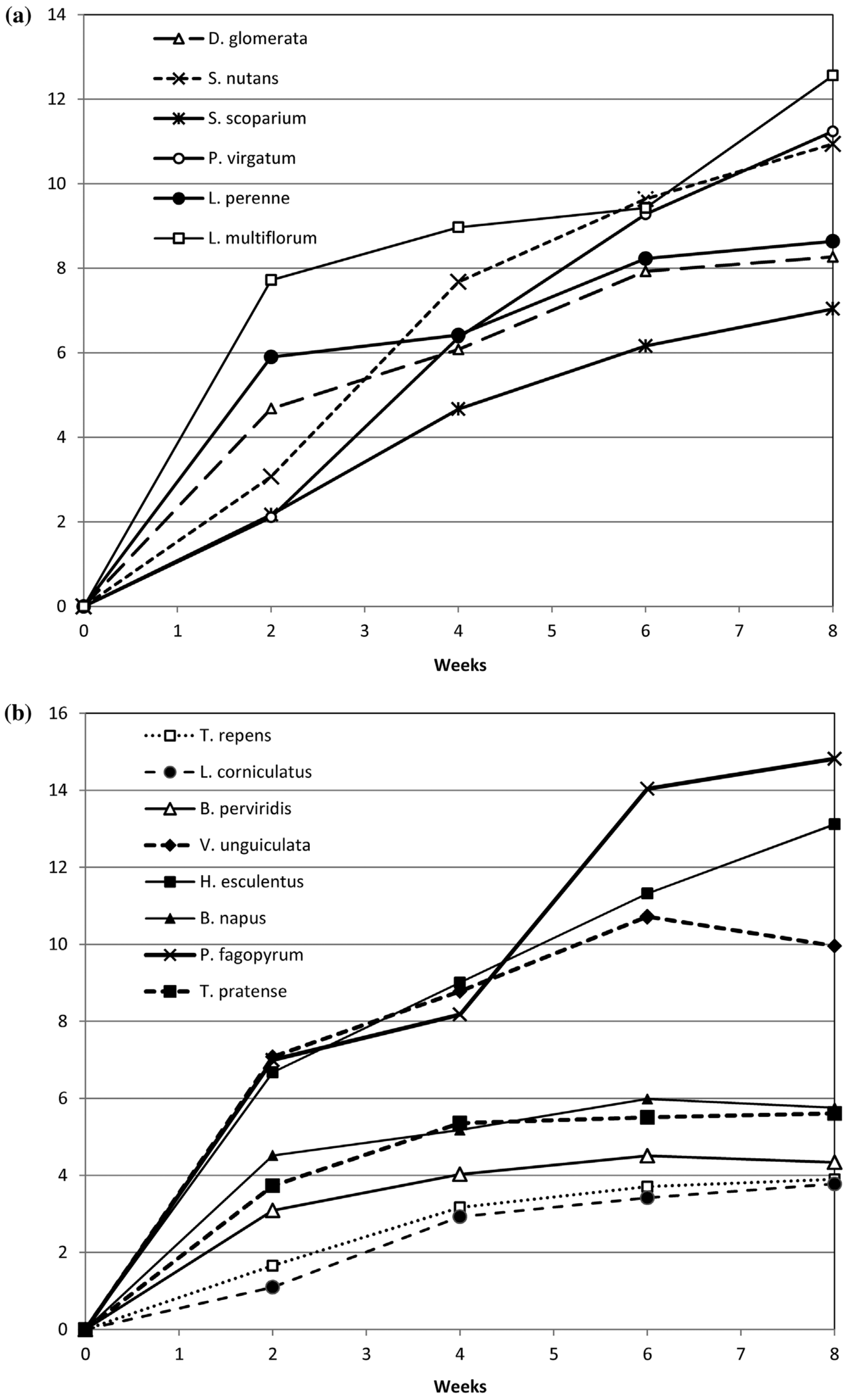

Figure 2. Height growth. Mean height of species over the first eight weeks after seeding, and averaged across soil types (a) monocot species, (b) dicot species.

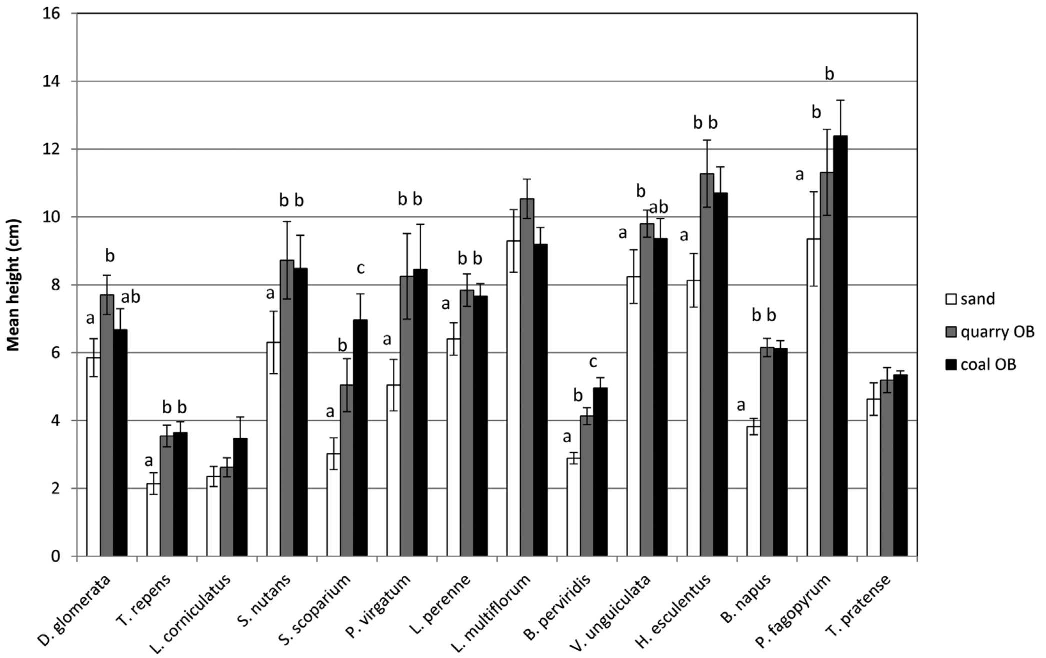

Figure 3. Plant height in three soil types. Mean plant height in vermiculite and quartz sand mixture at 1÷1 (v÷v) ratio, quarry overburden and coal overburden, averaged over the eight week study period. Bars indicate standard error. Different letters indicate differences between soil types for a given species at $p < 0.05$.

Transpiration rates were highly variable within species, but also differed greatly between species (Figure 4). Transpiration rates were very low in the five dicot species that showed stalled height growth, at less than half that of the three faster growing species. Grasses generally had much higher transpiration rates than the dicots, but was highly variable and did not appear to be related to growth rate. A significant effect of soil type was found in only two species: *B. perviridis* and *L. perenne*.

There was a significant difference in foliar pigment concentration among species (Figure 5), but no significant effect of soil type was found. Foliage of many species appeared to be chlorotic, and only the three Fabaceae species had relatively high concentrations of chlorophyll in foliage. Higher total chlorophyll a and b was shown at two Fabaceae, ie red clover (*Trifolium pratense* L.) and white clover (*Trifolium repens* L.). The fastest growing dicot, *P. fagopyrum*, had the lowest total chlorophyll concentration of all species tested.

Discussion

Reclaimed mined land is a highly variable and often challenging environment for the establishment of plants. Although sites differ greatly depending on climate, local geology and reclamation methods there are several characteristics that are commonly encountered on reclaimed mines: relatively low water availability due to coarsely textured soils with little or no organic content, and low nutrient availability. Soil texture and chemistry may differ substantially from local undisturbed soils, depending on the type of mining and methods of material placement, and soil microbial populations typical of native soils are absent. This means that in many cases, seeds naturally arriving on site may not contain species that are able to colonise a newly reclaimed site and seeding is necessary to speed the recovery of ecological processes.

Species that have been widely adopted for mine reclamation are likely to be ones that are tolerant of a wide range of environmental conditions, and in particular, those that are tolerant of low water

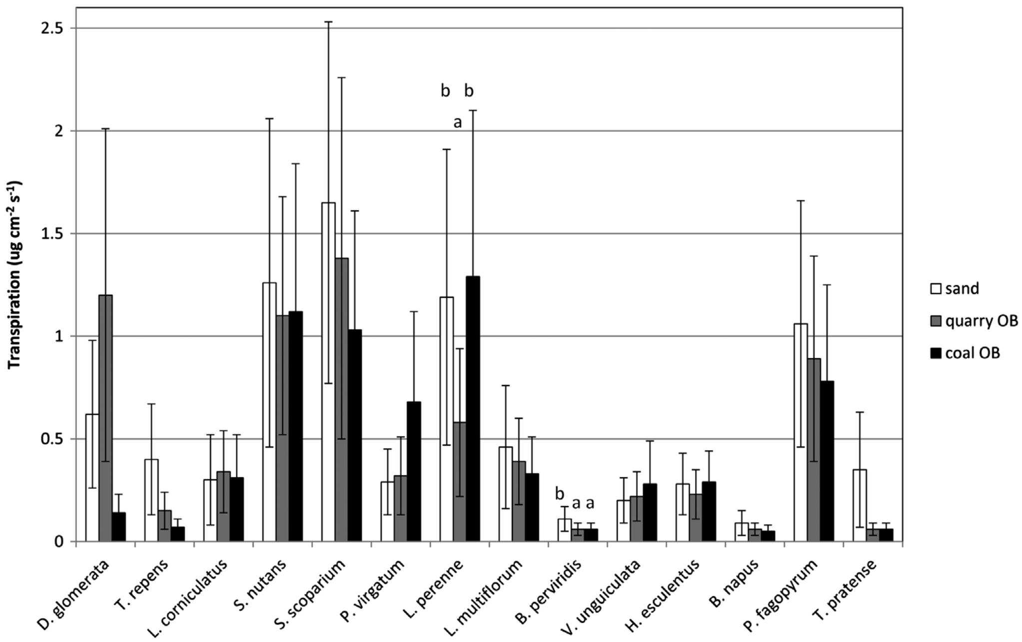

Figure 4. Transpiration rate in three soil types. Mean midday foliar transpiration rate of 14 species in vermiculite and quartz sand mixture at 1÷1 (v÷v) ratio, quarry overburden and coal mine overburden, measured 11 weeks after seeding. Bars indicate standard error. Different letters indicate differences between soil types for a given species at $p < 0.05$.

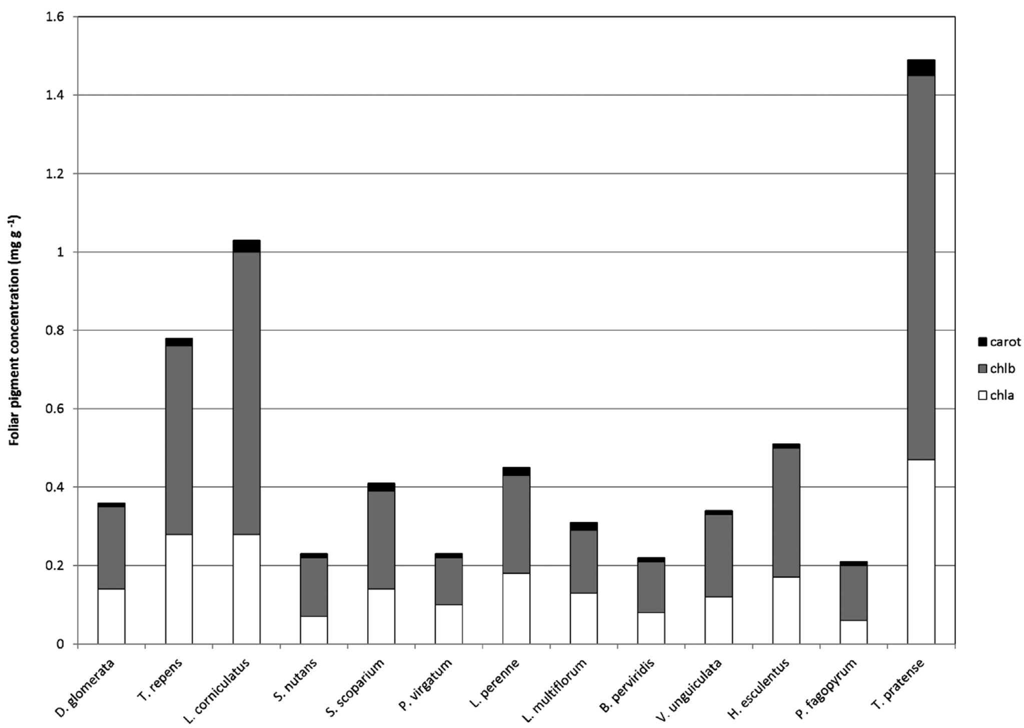

Figure 5. Foliar pigment concentration based on fresh weight of tissue, shown as the sum of chlorophyll a, cholorophyll b and total carotenoids. Data are averaged across three soil types for each species.

Table 4. Summary of traits potentially desirable in reclamation ground covers.

	Germ >30%	Cover >50% within 2 weeks	No germ × pH	No cover × soil type	No height × soil type	No transp × soil type	Low transp. rate	Foliar pigmen *t* > 0.4 mg/g
B. napus	√	√				√	√	
B. perviridis	√	√	√				√	
D. glomerata			√	√		√		
H. esculentus	√	√	√			√	√	√
L. multiflorum		√	√		√	√	√	
L. perenne	√		√	√				√
L. corniculatus			√	√		√	√	√
P. virgatum			√	√		√	√	
P. fagopyrum		√	√			√		
S. scoparium			√			√		√
S. nutans	√		√			√		
T. pratense	√	√	√	√	√	√	√	√
T. repens	√	√	√			√	√	√
V. unguiculata		√		√		√	√	

Notes: Average germination percentage of 14 species tested over a pH range from 5 to 10, and early growth and establishment tested on 3 soil types. No germination x pH indicates no significant effect of pH on germination at $p < 0.05$. No cover, height and transpiration × soil type indicate no significant interaction was found between those variables and soil type and ($p < 0.05$). Low transpiration rate indicates species that had mean transpiration rates below 0.05 ug cm^{-1} s^{-1} across all soil types.

and nutrient availability. Also likely to be important in the selection of species is its reliability of germination and establishment, and the rapid development of a vegetative cover to control erosion and microclimate below. These desirable characteristics, along with consistent performance across soil types, indicated by a lack of interaction between soil type and growth or physiology, are summarised in Table 4.

For water-stressed environments, the most drought-adapted species at the individual plant scale has the lowest daily transpirational water consumption [11]. Transpiration reduction also means increasing water use efficiency [12] which may show better adaptability in unfavourable soil conditions. However, we did not, under our test conditions, find lower transpiration rates in species commonly used for mine reclamation, and only two species showed transpiration rate to be related to soil type under our well-watered conditions. Other traits may be more important in conveying drought tolerance; for some species adaptation to drought may involve the ability of the plant to maintain high transpiration rates when not stressed [13], while other species may adapt to drought through morphological changes such as biomass partitioning [14] or leaf size [15] or possibly anatomical characters [6] rather than through physiological mechanisms.

Our results suggest that the average germination percentage is not an important factor in the adoption of species for mine reclamation. Some highly referenced species such as *D. glomerata* and *S. scoparium* have fairly low germination rates in this study. Low germination rates may be overcome by high seeding rates, and so may be acceptable for species with readily available and inexpensive seed. Because species mixtures are generally seeded, interactions and early development of seedlings are important, and can determine the success of other species in the mixture. One of the most widely used species, *D. glomerata*, has been found to dominate the vegetation and suppress the development of other species, despite a low germination rate [16]. As suggested by the authors of that study, the ability of this species to spread laterally once established may explain its dominance, as its early height growth and development of foliar cover were not among the most rapid of the species we tested.

Although rapid growth of vegetation is often cited as a desirable characteristic to control erosion on reclaimed sites, we found that the most frequently used species tended to have a moderate growth rate. As the growth of ground cover is important as it supports revegetation success [17], ground covers which perform consistently across soil types show their adaptability. While we expected the most frequently used species to be relatively insensitive to soil type, this was not found to be the case and *L. corniculatus* was the only species that performed consistently across all soil types, for the parameters measured. More nutrients in coal spoil than in quarry soil or in the vermiculite/sand mixture (Table 1), and/or soil texture may explain the plant growth differences (Figure 1). Except for sodium and potassium concentration, analysed minerals were lowest in vermiculite/sand, and nutrient deficiencies would be exacerbated by the high pH of this growth medium. Nutrient deficiency likely limited growth of these species tested in all of these soils, as suggested by foliar chlorophyll values towards the low end of reported values for herbaceous plants. Only *T. pratense* showed higher values that are within the typical reported range for that species [18]. Although faster growing species are often tolerant of low nutrient conditions, species with slower growth and less biomass turnover may dominate on low nutrient sites [19]. Better able to endure the stressful, low nutrient environments of mine sites, species of moderate growth rate may have been favoured for reclamation due to their ability to persist and spread.

It is also possible that the adoption of species for mine reclamation is driven to some extent by the results of early published research. A study undertaken in 1978 to evaluate the potential of 17 herbaceous species, as well as trees and shrubs for mine reclamation by rating their aggression, survival, and erosion control; *P. virgatum* and *L. corniculatus* were among the top ranked, while *T. pratense* was rated as 'fair' and has gained less widespread use [20]. Although this investigation was limited to biological characteristics, certainly seed cost and availability have also played a role in the adoption of species for use in reclamation, as is native status and public perception. It is hoped that in the future, the adoption of species will be based in greater part on their site suitability and biological characteristics, and ability to promote the development of maximum ecosystem functionality. Our results, that species of moderate growth rate and persistence are more readily adopted for use in reclamation, provides a starting point for further testing.

Conclusion

Our results suggest that plants with a moderate growth rate, which may be able to tolerate and persist in the low-nutrient environment of reclaimed mines, are more likely to be adopted for widespread use. One Fabaceae ground cover species, ie white clover (*Trifolium repens* L.), showed high potential as ground cover for reclaimed mines in the eastern United States.

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ORCID

Eddy Nurtjahya http://orcid.org/0000-0003-4641-069X

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