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# Composted Sewage Sludge Can Improve the Physiological Properties of *Betula schmidtii* Grown in Tailings

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**We investigated how the application of composted sewage sludge to tailings affects the physiological response of woody plants growing on abandoned coal-mining sites. Twenty seedlings of *Betula schmidtii* were transplanted to pots containing various combinations of artificial soil plus nursery soil, tailings, composted soil, or tailings amended with composted soil. Dry weights, shoot to root ratios, relative growth rates (RGR), chlorophyll content and fluorescence, and carbohydrate concentrations were assessed at the end of the experiment. Growth responses differed significantly among soil types. For example, dry weights were greatest for seedlings grown in composted soil and smallest for plants raised in pure tailings. Shoot to root ratios were higher for seedlings in composted soil compared with those in either tailings or nursery soil. Leaf chlorophyll content was twice as high for seedlings from composted soil than for those in the nursery soil or tailings; chlorophyll fluorescence (*Fv/Fm*) was lower for seedlings in either nursery soil or tailings than for those in composted soil. In contrast, plants grown in either nursery soil or tailings had higher starch concentrations in their stems, whereas the carbohydrate allocation of seedlings in composted soil was highest in the leaves, followed by stems and roots. Overall, the carbohydrate content was highest in the leaves, except for seedlings treated with tailings. Therefore, we believe that composted soil can improve the physiological and biochemical properties of trees growing in tailings when appropriate nutrients are supplemented.**

*Keywords: Betula schmidtii*, carbohydrate allocation, chlorophyll fluorescence, coalmine land, composted soil, relative growth rate

By the middle of the 1980s, coal had become the major energy resource in Korea, with most being taken from underground mines. This type of mining has now disturbed large regions of that country, leading to the loss of farmland acreage. Increased mining activity has also inevitably caused a rise in the amount of coal waste. In addition, environmental and social problems have arisen, including ground pollution, run-off, soil erosion, landslides, sodicification and desertification, unemployment, and severe conflicts between the farming and mining industries (Hu, 2000).

To solve these problems, the Coal Industry Promotion Board (CIPB) has conducted rehabilitation and revegetation projects on abandoned coalmine lands, at government expense. Although some efforts have been very successful, the implementation of conventional erosion control measures and techniques has not always been so effective (Woo, 2000). Ecological restoration and mine reclamation have become important components of sustainable development strategies in many countries (Gao et al., 1998). Good vegetative cover also benefits the remediation of con- taminated lands, thereby enhancing amenity values and preventing surface soil erosion (Baker et al., 1994). However, at many mining sites, adverse factors, such as acidity, nutrient deficiencies, toxic heavy-metal ions, and poor physical structure, interfere with the restoration of vegetation (Hossner and Hons, 1992; Pichtel and Salt, 1998). The use of native species that are tolerant of the poor growing conditions associated with these tailings may improve the success rate for reclamation and phytoremediation of these abandoned lands (Voeller et al., 1998).

Most sewage sludge contains >1% total nitrogen (Barnhisel, 1988), and can be used to compensate for N deficiencies found at mine sites. In addition, the pH and physical properties of tailings can be improved by the addition of such organic residues (Tester, 1990; Yum et al., 1999). However, these beneficial effects must be weighed against the risk of $NO_3$ or heavy-metal leaching. Therefore, researchers have evaluated the optimal amount of sewage sludge that can be applied for enhancing plant growth while minimizing groundwater pollution (Wilden et al., 2001). Likewise, the main objective of our study was to investi-

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gate how the application of composted sludge affects the physiological response of a native tree species *B. schmidtii*, growing in the tailings found on abandoned coalmine lands.

## MATERIALS AND METHODS

### Plant Material

*B. schmidtii* is a pioneer tree species on abandoned coalmine lands located at the Sododong in the Taebaek area of Korea (Lee et al., 2002). We collected seeds of this species from a wild population growing in tailings on this site. The seeds were sown in containers with vermiculite in early Spring 2002. Each container was irrigated immediately after sowing and then at two-week intervals.

### Soil Preparation

Various texture combinations of growing media were used to test the growth of *B. schmidtii* in the greenhouse (Table 1). Soil from nursery plots at the Korea Forest Research Institute, Suwon, served as the control. The other media included: 1) sewage sludge, from the Taebaek treatment plant, that was composted with several additives for approximately 45 d; 2) artificial soil, which consisted of 1:1:1 sand: peat moss: vermiculite (volume basis); and 3) tailings soil collected at the mining site. Predetermined ratios of artificial soil, sieved tailings, and composted soil were thoroughly mixed in plastic basins. We used 14- X 13-cm pots, into which two pieces of 0.1-mm plastic screen had been placed at the bottom to retain the fine particles of each soil type.

### Plant Growth Experiment and Analyses

At seven weeks after sowing, 20 seedlings of the same height (approx. 5 cm) were transplanted to individual pots (five replicates each for four treatments). The pots were arranged in a randomized block design in the greenhouse, watered from above daily, and moved about every two to three weeks throughout the 80-d experimental period to minimize positional effects. Temperatures varied between 17°C and 35°C, with a day length of 13~14 h. Seedling heights were recorded every week. At the end of the experiment, the relative growth rate (RGR), as indicated by height, was calculated according to: $[ln(x_2) - ln(x_1)]/(t_2 - t_1)$, where $x_1$ was the height at time $t_1$ (start of experiment) and $x_2$ the height at time $t_2$ (termination of experiment).

Leaf chlorophyll content was determined with a chlorophyll meter (SPAD-502, MINOLTA, JAPAN) when testing had concluded. *In vivo* chlorophyll fluorescence measurements also were made using a pulse amplitude modulation fluorometer (OS5-FL OPTI-SCIENCES, USA). We monitored fluorescence emissions from the interveinal regions on the upper surfaces of the youngest fully expanded and mature leaves. Prior to their measurements, the leaves were kept in the dark for 40 min before modulated light was turned on to obtain the dark-adapted minimal fluorescence ($F_0$). A saturating pulse (0.1 s) of light was also applied to obtain the dark-adapted maximal fluorescence ($F_m$). The ratio $F_v/F_m$ was then calculated, where $F_v$ (variable fluorescence in dark-adapted leaves) was equal to $F_m - F_0$.

For harvesting, the plants were carefully removed from their pots, washed with tap water to remove any attached soil particles, and rinsed three times with deionized water. Dry weights of the separated leaves, stems, and roots were then recorded. To determine their patterns of energy allocation, carbohydrate concentrations in the individual fractions were analyzed according to the glucose-oxidase method, as described by Hendry and Price (1993). In addition, total non-structural carbohydrates (TNC) were estimated as outlined by Paynter et al. (1992).

**Table 1.** Experimental design for pot trial.

| Treatment | Original samples mixed by v/v ratio |
|---|---|
| N (50) | 50% artificial soil[a] + 50% nursery soil[b] |
| T (50) | 50% artificial soil + 50% tailings[c] |
| T (25) + S (25) | 50% artificial soil + 25% tailings + 25% composted sewage sludge[d] |
| S (50) | 50% artificial soil + 50% composted sewage sludge |

[a]Mixture of 1:1:1 sand, peat moss, and vermiculite (v:v:v).
[b]Collected from Korea Forest Research Institute in Suwon.
[c]From abandoned coalmine lands at Taebaek.
[d]From Taebaek sewage sludge treatment plant.

### Data Analyses

The data were statistically analyzed according to the SAS System for Windows, Version 6.12 (SAS Institute, USA). Mean values per treatment were compared by ANOVA; when significant differences ($P \leq 0.05$) were indicated, Duncan's multiple range tests were performed.

## RESULTS AND DISCUSSION

### Growth Performance

Growth responses were significantly different ($P < 0.05$) for *B. schmidtii* seedlings raised in the various soil types, as seen by the leaf, stem, root, and total dry weights as well as their relative growth rates (Table 2; Fig. 1). For example, dry weights were greatest from seedlings grown in composted soil and smallest for those planted in the tailings. However, plant growth in tailings that also contained 25% composted soil did not differ significantly from those treated with 50% composted soil. Shoot to root ratios were higher for seedlings in the composted soil than in the tailings or nursery soil.

Municipal sewage sludge has been proposed as a suitable substrate for low-mineral and coarse soils. In our trial, composted soil containing sludge improved height growth. This addition also significantly changed seedling growth patterns. In general, plants allocate resources to the roots at the expense of shoot development in order to maximize the acquisition of scarce nutrients (Chapin, 1991). However, our seedlings in the composted soil showed significant increases in shoot rather than root weights, a response that matches the growth pattern normally seen for plants in a fertile habitat. Therefore, our study demonstrates that composted soil can improve the poor nutrient conditions ordinarily found with tailings.

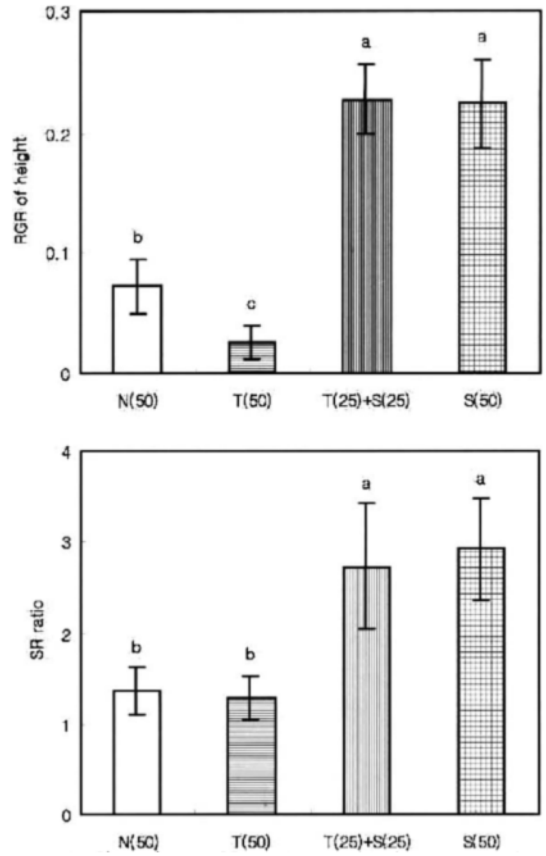


**Figure 1.** Shoot/root (SR) ratios and relative growth rates (RGR; by height) of *B. schmidtii* plants grown in nursery soil, tailings, or composted soil. Each data point represents mean of five replicates ± standard deviations. Different letters indicate significant differences among treatments at $p < 0.05$, by Duncan's multiple range tests.

Nevertheless, excessive applications of composted soil can potentially cause $NO_3$ concentrations to rise in the soil solution, risking an increase in heavy-metal leaching (Wilden et al., 2001). To prevent these problems, one must determine the appropriate amount to

**Table 2.** Dry weights measured from *B. schmidtii* leaves, stems, roots, and whole plants grown in various mixtures of nursery soil (N), tailings (T), and composted soil (S). Each value represents mean of five replicates ± standard deviations. Different letters within the same column indicate significant differences among treatments at $p < 0.05$ by Duncan's multiple range tests.

| Treatment | Dry weight (g) | | | |
|---|---|---|---|---|
| | Leaf | Stem | Root | Total |
| N(50) | $0.25 \pm 0.08^{b}$ | $0.11 \pm 0.03^{c}$ | $0.26 \pm 0.05^{b}$ | $0.62 \pm 0.15^{b}$ |
| T(50) | $0.08 \pm 0.02^{c}$ | $0.02 \pm 0.01^{c}$ | $0.08 \pm 0.02^{c}$ | $0.19 \pm 0.03^{c}$ |
| T(25) + S(25) | $1.08 \pm 0.33^{a}$ | $0.58 \pm 0.17^{b}$ | $0.65 \pm 0.31^{a}$ | $2.31 \pm 0.74^{a}$ |
| S(50) | $1.10 \pm 0.27^{a}$ | $0.70 \pm 0.24^{a}$ | $0.63 \pm 0.18^{a}$ | $2.44 \pm 0.64^{a}$ |
| Pr > F | 0.0001 | 0.0001 | 0.0001 | 0.0001 |

be applied to prevent nutrient leaching while enhancing plant growth. Based on our results, we suggest that application rates of < 25% composted soil are sufficient for achieving a positive effect on biomass production in *B. schmidtii*.

## Chlorophyll Content and Chlorophyll Fluorescence

Chlorophyll contents differed significantly among the seedlings grown in the various soil types. SPAD values in the leaves of plants in composted soil were twice as high as those measured in seedlings raised in nursery soil or tailings (Fig. 2). This larger chlorophyll content reflects the contribution that composted soil makes in increasing chlorophyll synthesis when one compensates for an inherent nitrogen deficiency.

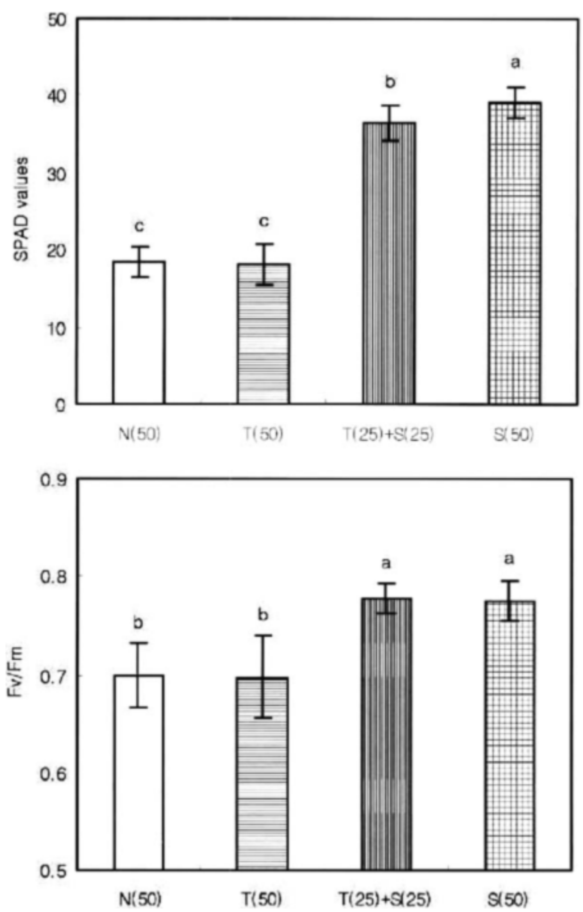


**Figure 2.** Chlorophyll content (upper) and chlorophyll fluorescence response (lower) in leaves of *B. schmidtii* plants grown in nursery soil, tailings, or composted soil. Each value represents mean of five replicates ± standard deviations. Different letters within the same column indicate significant differences among treatments at $p < 0.05$, by Duncan's multiple range tests.

Chlorophyll fluorescence in the leaves also differed significantly ($P < 0.05$) among treatments (Fig. 2). The maximal photochemical yield ($F_v/F_m$) was lower for seedlings in nursery soil or tailings than for those planted in 25% or 50% composted soil. The ratio of variable to maximal fluorescence ($F_v/F_m$), which represents the maximal photochemical yield of PSII centers (Butler and Kitajima, 1975), is highly correlated with the quantum yield of net photosynthesis for intact leaves exposed to various degrees of photo-inhibition (Demmig and Bjorkman, 1987). PSII is a known primary target of photo-inhibition, thereby lowering not only PSII quantum yield but also the capacity for PSII-driven electron flow (Baker, 1991).

Because the value of $F_v/F_m$ is determined by the photochemical efficiency of open (oxidized) PSII, several stress factors impair PSII function in both mature and young leaves. We observed here that the changes in chlorophyll fluorescence in leaves of seedlings grown in tailings were consistent with those normally associated with poor soil conditions, with the effect on photosynthetic performance being only negligible. The lower $F_v/F_m$ ratio for seedlings treated with tailings indicated photo-inhibitory damage to the PSII reaction centers, a result of nutrient deficiency, coarse soil texture, and etc.

## Patterns of Carbohydrate Allocation

Carbohydrate concentrations in the tissues of *B. schmidtii* showed significant differences among soil types and plant organs (Fig. 3). In particular, seedlings grown in nursery soil or tailings had remarkably higher starch contents in their stems compared with those treated with composted soil.

In contrast, total carbohydrate content did not vary as greatly among organ types for seedlings in nursery soil or tailings. However, TNC contents from seedlings treated with composted soil were highest in the leaf, followed by the stem and roots (Fig. 4). Carbon allocation was greatest in the leaves for all soil combinations except the tailings treatment. We especially observed clear differences in levels for leaves of seedlings grown in composted soil (Fig. 4). This indicates that seedlings in that soil type invest more energy in aboveground growth, while performance by the tailings-treated seedlings is restrained by a shortage of energy.

In general, factors that influence leaf area development and duration, as well as the conversion efficiency of intercepted light into biomass, are critical

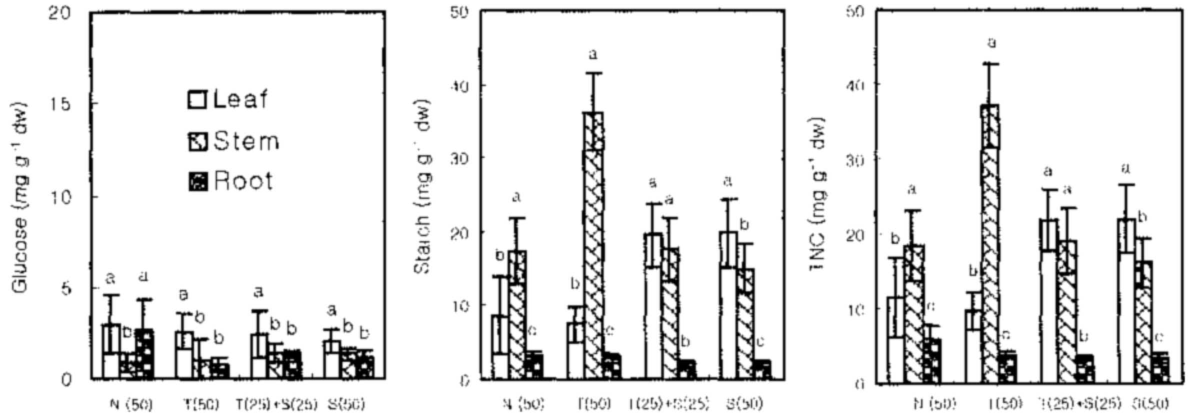


**Figure 3.** Changes in carbohydrate concentration within leaves of *B. schmidtii* plants grown in nursery soil, tailings, or composted soil. Each data point represents mean of five replicates ± standard deviations. Different letters within the same treatme nt indicate significant differences among organs at $p < 0.05$, by Duncan's multiple range tests.

for plant production (Cannell et al., 1988). One that is of central importance in this context is plant-nitrogen status. A larger fraction of assimilates is used aboveground when N status is improved (Ingestad, 1979), a response we also saw when composted soil was applied to our seedlings.

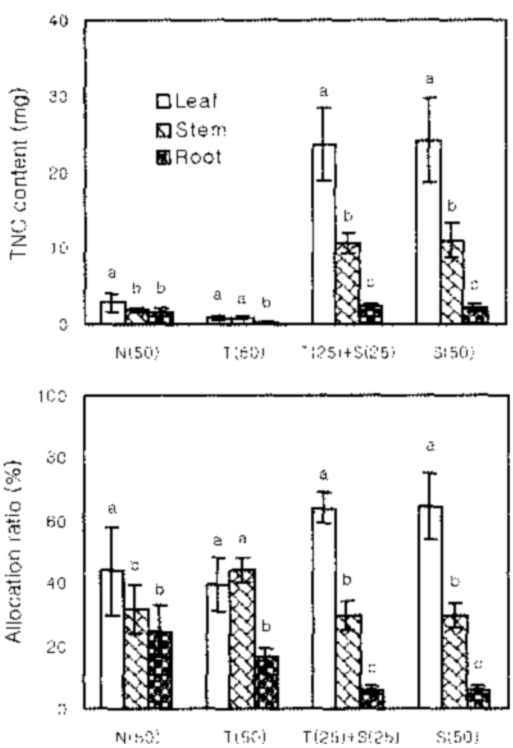


**Figure 4.** Changes in leaf carbohydrate contents and allocation ratio among organs of *B. schmidtii* plants grown in nursery soil, tailings, or composted soil. Each data point represents mean of five replicates ± standard deviations. Different letters within the same treatment indicate significant differences among organs at $p < 0.05$, by Duncan's multiple range tests.

In contrast, by increasing the fraction of assimilates used for root growth in response to decreased N availability, plants can partly counterbalance a troublesome soil-N situation. Though not always true, it is logical to expect that the lack of any mineral nutrient would increase the root:shoot ratio (Thornley, 1972; Dewar, 1993). Nevertheless, recent experiments performed at steady-state nutrition have shown that an increase in the root fraction is obtained only with plants grown under limited N, P, or S (Ericsson and Kähr, 1995). Moreover, when root growth is suppressed by the reduced availability of K, Mg or Mn, it always coincides with a decrease in the leaf storage pool of non-structural carbohydrates. In addition, when plants are low in non-structural carbohydrates, root growth is more strongly suppressed than the activity of the apical shoot meristems (Ericsson et al., 1996).

In our trial, the deficiency of nutrients in the tailings soil caused seedling growth to be restrained due to a disturbance in their physiological metabolism. However, adequate nutrient levels in our composted soil were sufficient to improve growth and overcome such physiological damage. Therefore, we believe that continuously supplementing the soil on abandoned coalmine lands with composted sewage sludge can improve the physiological and biochemical properties of naturally introduced pioneer trees or transplanted trees in order to restore those sites. In addition, improved aboveground growth of seedlings will contribute to rapid revegetation on the tailings surfaces while protecting the soil from excessive leaching of minerals and toxic elements.

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