Physiological and Morphological Traits and Competence for Carbon Sequestration of Several Green Roof Plants under a Controlled Environmental System

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ABSTRACT. The physiological and morphological traits of green roof plants are key to understanding the environmental benefits of green roofs. However, the comparative investigation of physiological and morphological traits of green roof plants is limited. Moreover, there have been few studies on the relevance of physiological and morphological traits and competence for carbon sequestration of green roof plants. In particular, *Sedum* L. species are generally regarded as crassulacean acid metabolism (CAM) plants, but several *Sedum* species are recognized as having an “inducible type of CAM.” These plants are C₃ and C₄ plants, with the ability to switch their carbon metabolism to the CAM pathway. In the case of *Sedum*, the inducer of CAM is drought stress. This observation suggests that differences in water regimes result in physiological and morphological changes that may have a considerable effect on the environmental benefits of *Sedum* green roofs. The purpose of this study is to compare the physiological and morphological traits of four green roof plants and discuss the influence of these traits on their environmental benefits under a controlled environmental system. In addition, we attempted to clarify the relevance of physiological and morphological traits and competence for carbon sequestration in each plant using growth analysis. We used *Sedum mexicanum* Britton, *Sedum aizoon* L., *Zoysia matrella* (L.) Merr., and *Ophiopogon japonicus* (Thunb.) Ker Gawl. in this study, and only the two *Sedum* species were assigned treatments with different frequency of irrigation to investigate physiological and morphological responses and variation in carbon sequestration. The two *Sedum* species exhibited the C₃ photosynthetic pathway in wet and dry treatments, implying the inducible type of CAM. Morphological responses of the two *Sedum* species were significantly affected by the different frequencies of irrigation; plants responded to increases in below-ground resources (water and nutrients) with increased biomass allocation of leaves and stems. Owing to these physiological and morphological responses in the wet treatment, transpiration rates and competence for carbon sequestration [relative C sequestration rate per whole-plant C content (RGR_c)] of the two *Sedum* species were higher than those of *Z. matrella* and *O. japonicus*. This result suggested that the cooling effect and carbon sequestration of *Sedum* in wet and increased nutrient conditions are equivalent to those observed in other plants. In addition, the net assimilation rate (NAR_c) and leaf area ratio per whole-plant C content (LAR_c) were significantly correlated with photosynthetic rates and biomass allocation. Thus, it became clear that growth analysis can reveal the relevance of physiological (NAR_c) and morphological (LAR_c) traits and RGR_c. Our results will serve as a baseline of the physiological and morphological traits and carbon sequestration of green roof plants and contribute to more suitable design and maintenance of vegetation in green roofs.

The green roof is known to be a beneficial technology in urban environments. Some of the benefits include cooling and insulation of buildings (Sailor, 2008; Wong et al., 2003), mitigation of the urban heat-island effect (Susca et al., 2011), stormwater management (Getter et al., 2007; Villarreal and Bengtsson, 2005), carbon sequestration and air pollution reduction (Getter et al., 2009; Yang et al., 2008), and habitat provision for other organisms (Kadas, 2006). Owing to these environmental benefits, the green roof has been adopted in many countries. These benefits depend on the presence of living plants and the growing medium. For example, the boundary layer of air over the roof is cooled by evapotranspiration,

shading from plants, and the trapping of cool air by the vegetation layer (Dimoudi and Nikolopoulou, 2003; Solecki et al., 2006). In addition, air pollution is reduced through plant photosynthesis and trapping of the gases by vegetation and soil layers (Garland, 1977). Consequently, the physiological and morphological traits of the plants are key to understanding the environmental benefits of green roofs. However, the comparative investigation of physiological and morphological traits of green roof plants is limited (Lundholm et al., 2015). Moreover, there have been few studies examining the relevance of physiological and morphological traits and competence for carbon sequestration in green roof plants.

Warm-season turfgrasses and *Sedum* species are the most common vegetation in Japan. Green roofs composed of warm-season turfgrasses or other perennials are generally installed

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with irrigation systems to prevent drought stress. In contrast, *Sedum* green roofs are not always installed with irrigation because these plants can adapt to periodic drought. They are regarded as CAM plants whose stomata remain closed during the day, with gas exchange occurring at night (VanWoert et al., 2005). This physiological pathway plays a crucial role in the plant's response to drought stress; other CAM plants have also been investigated for utilization in green roofs (Lin and Lin, 2011). In addition, several *Sedum* species are recognized as having an "inducible type of CAM" (Gravatt and Martin, 1992; Lee and Griffiths, 1987). They are C₃ and C₄ plants with the ability to switch their carbon metabolism to the CAM pathway. In the case of *Sedum*, the inducer of CAM is drought stress (Sayed, 2001). This observation suggests that differences in water regimes result in physiological and morphological changes that may have a considerable effect on the environmental benefits of *Sedum* green roofs (e.g., carbon sequestration, air pollution reduction, and cooling effects). Further, although a *Sedum* green roof is likely to be exposed to drought conditions, the substrate may be wet during the rainy season in southeast Asian countries (Chen, 2013). Van Mechelen et al. (2015) also suggested that irrigation is necessary on green roofs in (semi)-arid climates for the survival and success of extensive green roof plantings. Therefore, it is necessary to investigate the physiological and morphological responses of *Sedum* to various water conditions resulting from different maintenance practices and climates.

The purpose of this study is to compare the physiological and morphological traits of several green roof plants and discuss the influence of these traits on their environmental benefits. In addition, we attempted to clarify the relevance of physiological and morphological traits and competence for carbon sequestration in each plant using growth analysis. In particular, *Sedum* species were assigned different water regimes to investigate the physiological and morphological responses and variation in carbon sequestration.

Materials and Methods

Plant material

We focused on groundcover herbaceous green roof plants for comparison of physiological and morphological traits; thus, *S. mexicanum*, *S. aizoon*, *Z. matrella*, and *O. japonicus* 'Tama-Ryu' were selected for this study. Although these species are widely distributed in Japan, the photosynthetic pathway of the two *Sedum* species is not well understood. *Zoysia matrella*, a warm-season turfgrass and C₄ plant, is one of the most common green roof plants in Japan. *Ophiopogon japonicus* is an evergreen perennial.

All plants in this study were propagated as cuttings in plug flats (128 cells/tray) filled with seedling propagation medium (Metro Mix; Sun Gro Horticulture, Agawam, MA). After ≈1 month, plugs were planted in 0.2-L polyethylene pots (44 cm²) filled to a depth of 5 cm with commercial artificial soil for green roofs (114 mg·kg⁻¹ NO₃-N, 323 mg·kg⁻¹ NH₄-N, 159 mg·kg⁻¹ P₂O₅, 32 mg·kg⁻¹ K₂O, 41 mg·kg⁻¹ CaO, and 2 mg·kg⁻¹ MgO). The artificial soil composition was 75% perlite, 22% bark and peat, and 3% zeolite. Bulk density of the substrate was 0.22 g·mL⁻¹, and three-phase distribution was solid phase of 37.0%, liquid phase of 49.4%, and gas phase of 13.6%. Because it is lightweight and has high water retention capacity, such green roof soils, which mainly consist of a perlite, are widely used for

extensive green roofs in Japan. The plants were placed in a totally controlled environmental system (phytotron) and grown about 3 weeks under the following conditions: 25 °C (light period) and 20 °C (dark period), 65% ± 10% humidity, 400 μL·L⁻¹ CO₂ concentration, 14 h light (200–250 μmol·m⁻²·s⁻¹), and 10 h dark.

Comparative study of physiological traits

EXPERIMENTAL DESIGN. To investigate the photosynthetic pathway of the two *Sedum* species in wet and dry conditions, individuals of the two *Sedum* species were assigned randomly to wet or dry treatments and acclimatized to each treatment for 3 weeks. Plants in the wet treatment were watered every day, and plants receiving the dry treatment were watered once per week. *Zoysia matrella* and *O. japonicus* only received the wet treatment, in accordance with general cultivation practice. In this study, to reduce the influence of nutrient-poor conditions on plant growth, an irrigation system supplied not water but nutrient solution (72 μL·L⁻¹ NO₃-N, 11 μL·L⁻¹ NH₄-N, 100 μL·L⁻¹ P₂O₅, 167 μL·L⁻¹ K₂O, 71 μL·L⁻¹ CaO, and 17 μL·L⁻¹ MgO) for 30 min by bottom watering in every treatment. All studies were carried out in the phytotron.

PHOTOSYNTHETIC PATHWAY OF *S. MEXICANUM* AND *S. AIZOON*. The photosynthetic and transpiration rates of five plants per treatment were measured for 4 h (2 h light period, 2 h dark period) using a photosynthesis system (LI-6400; LI-COR, Lincoln, NE) with an light-emitting diode light source. Measurement conditions were as follows: 25 °C (light) and 20 °C (dark), 50% ± 10% humidity, 400 μL·L⁻¹ CO₂ concentration, and 1000 μmol·m⁻²·s⁻¹ photosynthetic photon flux density (assumed roof conditions). The soil water content for individuals in the dry treatment was investigated after irrigation (0, 1, 2, 3, 4, 5, and 6 d) using a soil moisture sensor (EC-5 and ProCheck; Decagon, Pullman, WA).

COMPARISON OF PHOTOSYNTHETIC AND TRANSPIRATION RATES IN WET TREATMENT. For individuals of the four species in the wet treatment, photosynthetic and transpiration rates were measured at 15, 25, and 35 °C, which are assumed roof conditions. Other measurement conditions were the same as above. Photosynthetic water use efficiency [WUE_{ph} (photosynthetic rate/transpiration rate)] was calculated for each plant.

Comparative study of morphological traits and competence for carbon sequestration

EXPERIMENTAL DESIGN. To examine morphological traits and carbon sequestration, we investigated biomass allocation and whole-plant carbon sequestration in the phytotron.

In all four species, 15 total pots per species were selected at random (three pots were chosen at 0, 1, and 2 d, and six pots were selected at 3 d after the start of the experiment). After these samples were selected, the *Sedum* species were divided into wet, dry, and nonirrigation treatments. Plants in all treatments were irrigated at the start of the experiment (0 d). After this irrigation, the nonirrigation treatment was never irrigated. Wet and dry treatments and the irrigation system (supplied nutrient solution) were the same as above, but there was no irrigation in the wet treatment for 1–3 d. *Zoysia matrella* and *O. japonicus* plants only received the wet treatment.

Three pots were randomly harvested once per week for 4–6 weeks after the start of the experiment (28, 35, and 42 d), and the remaining six pots were harvested at the end of the experiment (49 d). We regarded these 15 total pots (at 28, 35,

42, and 49 d) as the result of treatment. For all samples, roots were removed from the soil and washed with distilled water.

MORPHOLOGICAL RESPONSES TO WATER REGIMES OF TWO SEDUM SPECIES AND COMPARISON TO *Z. MATRELLA* AND *O. JAPONICUS*. Plants were divided into leaves, stems, roots, and flowers and dried at 70 °C for 72 h. Before the leaves were dried, they were scanned (LP-A500; Epson, Suwa, Japan) and image analysis software ImageJ (Schneider et al., 2012) was used to measure the leaf area and greenness of leaves. We calculated the leaf area index [LAI (leaf area per 44 cm²)], and described greenness of leaves with G/R, which is related to chlorophyll content (Adamsen et al., 1999). Biomass allocation was analyzed using five indices: leaf mass fraction [LMF (leaf mass per whole-plant mass)], stem mass fraction [SMF (stem mass per whole-plant mass)], root mass fraction [RMF (root mass per whole-plant mass)], flower mass fraction [FMF (flower mass per whole-plant mass)], and specific leaf area [SLA (leaf area per leaf mass)].

QUANTITATIVE ANALYSIS OF CARBON SEQUESTRATION AND THE INFLUENCE OF PHYSIOLOGICAL AND MORPHOLOGICAL TRAITS USING GROWTH ANALYSIS. Plants carbon concentration was measured using an organic elemental analyzer (2400 SeriesII CHNS/O System; PerkinElmer, Waltham, MA); whole-plant carbon

content was quantified by multiplication of carbon concentration and whole-plant mass.

Growth analysis is a simple model that associates a plant's relative growth rate (RGR) with physiological [net assimilation rate (NAR)] and morphological [leaf area ratio (LAR)] traits (Lambers et al., 1989), which have a mathematical relationship ($RGR = NAR \times LAR$). In addition, growth analysis provides a more informative comparison of a plant's relative performance because it can reduce the influence of differences in the initial size and mass among the experimental plants (Hunt, 1982). This model is generally applied to dry weights, but this experiment focused on whole-plant carbon content because our goal was to examine the relevance of physiological and morphological traits and carbon sequestration in each plant. Thus, we carried out growth analysis using a functional approach (Hunt, 1982) and calculated relative C sequestration rate per whole-plant C content (RGR_c), NAR_c , and LAR per whole-plant C content (LAR_c). We used RGR_c as the measure of the ability to sequester carbon. Moreover, for the two *Sedum* species, growth response coefficients (GRC) of NAR_c and LAR_c (comparisons: wet and dry, wet and nonirrigation, and dry and nonirrigation) were calculated. The GRC values of NAR and LAR sum to 1.0, indicating the relative contribution

Fig. 1. Representative values of sequential photosynthetic rates of two *Sedum* species (*Sedum mexicanum* and *Sedum aizoon*) for 4 h in wet treatment (plants supplied nutrient solution every day) and dry treatment (plants supplied nutrient solution once per week) at 6 d after irrigation. Black bars indicate dark period.

Fig. 2. Mean values for photosynthetic rate of two *Sedum* species [(A) *Sedum mexicanum*, (B) *Sedum aizoon*] in dry (plants supplied nutrient solution once per week) and wet treatments (plants supplied nutrient solution every day). L and D indicate light and dark periods. SWC is the mean soil water content. Means $\pm$ SE are presented ($n = 5$). Significant differences among means are indicated by different letters based on Tukey-b test at $P < 0.05$.

of those parameters to a change in RGR (Poorter and Nagel, 2000; Poorter and Van der Werf, 1998). For example, if GRC_{LAR} is 1, a change in RGR is attributed only to a change in LAR. In other words, GRC_{NARc} and GRC_{LARc} indicate the influence of physiological and morphological changes on competence for carbon sequestration.

Statistical analysis

Data were analyzed using IBM SPSS Statistics (version 22.0; IBM Japan, Tokyo, Japan). Analysis of variance was used to assess the effects of treatments and the plant species. Differences in mean values were assessed with Student's *t* test or multiple comparisons [Tukey-b (homoscedasticity assumed) or Dinnett-T3 (homoscedasticity not assumed)].

Results and Discussion

Comparative study of physiological traits

PHOTOSYNTHETIC PATHWAY OF *S. MEXICANUM* AND *S. AIZOON*. Individuals of *S. mexicanum* and *S. aizoon* in the wet treatment exhibited CO₂ uptake only during the light period and no dark period CO₂ uptake in any of the samples (Fig. 1). In the dry treatment, *S. mexicanum* and *S. aizoon* did not exhibit CO₂ uptake during light and dark periods at 6 d after irrigation, similar to the photosynthetic pathway of several *Sedum* species which had an inducible type of CAM under drought conditions (Gravatt and Martin, 1992; Lee and Griffiths, 1987). The mean photosynthetic rate during the light period under the wet treatment was significantly higher than that under the dry treatment (Student's *t* test at $P < 0.05$). Thus, these results imply that *S. mexicanum* and *S. aizoon* have an inducible type of CAM.

Soil water content in the dry treatment was below 10% at 2 to 6 d after irrigation; however, *S. mexicanum* displayed the C₃ photosynthetic pathway (Fig. 2A). Moreover, although the frequency of water and nutrient supply under the dry treatment was lower than that under the wet treatment, photosynthetic and transpiration rates at 0 to 4 d after irrigation were not significantly different from those in the wet treatment (Fig. 2A; Table 1). *Sedum aizoon* also exhibited the C₃ photosynthetic pathway in the dry treatment (Fig. 2B), but the photosynthetic and transpiration rates were significantly lower than those in the wet treatment (Fig. 2B; Table 1). In green roofs, some studies suggested that *Sedum* species have exhibited the C₃ photosynthetic pathway (Butler and Orians, 2011; Durhman et al., 2006). Our results are consistent with this photosynthetic response occurring in green roofs.

COMPARISON OF PHOTOSYNTHETIC AND TRANSPIRATION RATES IN WET TREATMENT. The photosynthetic rate of *S. mexicanum* at all temperatures was significantly lower than that of *Z. matrella* and not significantly different from that of *O. japonicus* (Fig. 3). Photosynthetic rates of *S. aizoon* were significantly higher than those of *O. japonicus* and similar to those of *Z. matrella* except at 35 °C (Fig. 3). *Sedum* species in the wet treatment exhibited similar photosynthetic rates to those of other green roof plants,

including the C₄ plant, *Z. matrella*. However, the photosynthetic rate of C₄ plants is generally higher at high temperatures, so *Z. matrella* should show the highest photosynthetic rate at 35 °C.

Transpiration rates of the two *Sedum* species at each temperature were higher than that of *Z. matrella*, and there were significant differences from transpiration rates of *O. japonicus* at 15 and 25 °C (Table 2). WUE_{ph} of the two *Sedum* species was lower than that of *Z. matrella* and *O. japonicus* (Table 2). Although CAM plants typically have high WUE and low transpiration rates (Nobel, 1991), the two *Sedum* species in the wet treatment had distinctively lower WUE and higher transpiration rates than did other green roof plants. This result supports the C₃ pathway being used for photosynthesis.

Previous research has suggested that evapotranspiration is the most important contributor toward the cooling effect in green roofs (Feng et al., 2010; Oberndorfer et al., 2007; Takakura et al., 2000). In addition, some studies reported that irrigation has enhanced the cooling effect (Blanusa et al., 2013;

Table 1. Mean values (n = 5) for transpiration rates of two *Sedum* species (*Sedum mexicanum* and *Sedum aizoon*) in dry treatment (plants supplied nutrient solution once per week) and wet treatment (plants supplied nutrient solution every day).

Treatment	Time after irrigation (d)	<i>S. mexicanum</i>		<i>S. aizoon</i>	
		[mean ±SE (mmol·m ⁻² ·s ⁻¹ H ₂ O)]			
Dry	0	1.11 ± 0.15	c ^z	0.72 ± 0.17	ABC ^y
	1	1.30 ± 0.21	c	0.44 ± 0.07	C
	2	0.98 ± 0.23	bc	0.28 ± 0.03	C
	3	0.86 ± 0.20	bc	0.26 ± 0.04	BC
	4	0.63 ± 0.11	abc	0.04 ± 0.02	A
	5	0.31 ± 0.09	ab	0.01 ± 0.03	A
	6	0.14 ± 0.11	a	0.07 ± 0.03	AB
Wet	—	0.96 ± 0.21	bc	2.78 ± 0.24	D

^zSignificant differences among means are indicated by different letters based on Tukey-b test at $P < 0.05$.

^ySignificant differences among means are indicated by different letters based on Dunnett-T3 at $P < 0.05$.

Fig. 3. Means values for photosynthetic rates of four green roof plants (*Sedum mexicanum*, *Sedum aizoon*, *Zoisia matrella*, and *Ophiopogon japonicus*) in wet treatment (plants supplied nutrient solution every day) at each temperature (15, 25, and 35 °C). Means ± SE are presented (n = 5). Significant differences among means are indicated by different letters based on Tukey-b test at $P < 0.05$.

Table 2. Mean values (n = 5) for transpiration rates and photosynthetic water use efficiency (WUE_{ph}) of four green roof plants (*Sedum mexicanum*, *Sedum aizoon*, *Zoysia matrella*, and *Ophiopogon japonicus*) in the wet treatment (plants supplied nutrient solution every day) at each temperature (15, 25, and 35 °C).

Species	Transpiration rate						WUE _{ph}					
	[mean ±SE (mmol·m ⁻² ·s ⁻¹ H ₂ O)]						[mean ±SE (μmol·m ⁻² ·s ⁻¹ CO ₂ /mmol·m ⁻² ·s ⁻¹ H ₂ O)]					
	15 °C	25 °C	35 °C	15 °C	25 °C	35 °C	15 °C	25 °C	35 °C	15 °C	25 °C	35 °C
<i>S. mexicanum</i>	1.2 ± 0.2	bc ^z	1.6 ± 0.2	B ^z	2.3 ± 0.5	a'b' ^z	3.8 ± 0.8	a ^z	2.8 ± 0.4	A ^z	1.9 ± 0.3	a' ^y
<i>S. aizoon</i>	1.6 ± 0.3	c	2.4 ± 0.4	B	3.2 ± 0.6	b'	6.9 ± 0.3	a	5.0 ± 0.2	B	3.5 ± 0.3	a'
<i>Z. matrella</i>	0.7 ± 0.1	ab	1.2 ± 0.1	B	2.1 ± 0.1	a'b'	12.9 ± 0.6	b	11.5 ± 1.0	C	8.3 ± 1.0	b'
<i>O. japonicus</i>	0.2 ± 0.0	a	0.8 ± 0.1	A	1.0 ± 0.2	a'	9.6 ± 2.3	ab	6.1 ± 1.1	AB	3.8 ± 0.9	a'

^zSignificant differences among means are indicated by different letters based on Dunnett-T3 at *P* < 0.05.

^ySignificant differences among means are indicated by different letters based on Tukey-b test at *P* < 0.05.

Table 3. Mean values (n = 6) for dry weight, leaf area index (LAI), and the ratio of green to red pixels in an image of leaves (G/R) of two *Sedum* species (*Sedum mexicanum* and *Sedum aizoon*) in wet treatment (plants supplied nutrient solution every day), dry treatment (plants supplied nutrient solution once per week), and nonirrigation treatment [plants only supplied nutrient solution at the start of the experiment (0 d)] at the end of the experiment (49 d), and those of two green roof plants (*Zoysia matrella* and *Ophiopogon japonicus*) in the wet treatment.

Species	Treatment	Dry wt [mean ±SE (g)]	LAI (mean ± SE)	G/R (mean ± SE)
<i>S. mexicanum</i>	Wet	9.1 ± 0.4	C ^z	50.8 ± 1.3
	Dry	2.9 ± 0.1	B	11.3 ± 0.5
	Nonirrigation	0.7 ± 0.0	A	1.3 ± 0.1
<i>S. aizoon</i>	Wet	9.5 ± 0.5	c ^y	15.7 ± 0.7
	Dry	2.2 ± 0.1	b	4.4 ± 0.1
	Nonirrigation	0.7 ± 0.0	a	1.1 ± 0.0
<i>Z. matrella</i>	Wet	7.5 ± 0.5		17.3 ± 0.3
<i>O. japonicus</i>	Wet	1.9 ± 0.3		3.2 ± 0.4

^zSignificant differences among means are indicated by different letters based on Tukey-b test at *P* < 0.05.

^ySignificant differences among means are indicated by different letters based on Dunnett-T3 at *P* < 0.05.

Lin and Lin, 2011; Solecki et al., 2006). Therefore, our results indicate that the cooling effect of *Sedum* in wet and increased nutrient conditions may be equivalent to that of other plants from the standpoint of evapotranspiration. Moreover, even *Sedum* roofs with a low frequency of irrigation or rain events may improve the thermal load because *Sedum* in the dry treatment exhibited the C₃ pathway.

Comparative study of morphological traits and competence for carbon sequestration

PLANT STATUS. There was no significant difference in initial dry weight (±SE) between *S. mexicanum* (0.52 ± 0.03 g), *S. aizoon* (0.55 ± 0.03 g), and *Z. matrella* (0.50 ± 0.04 g). At the end of the experiment, the two *Sedum* species had significant differences in dry weight and LAI among the treatments (Table 3). The dry weight of *Z. matrella* was less than that of *Sedum* in the wet treatment. *Ophiopogon japonicus* had a lower dry weight than *Sedum* in the dry treatments, although its initial dry weight (0.66 ± 0.02 g) was significantly higher than that of the other species (Tukey-b test at *P* < 0.05). Under our experimental conditions, the LAI of *S. mexicanum* in the wet treatment was much larger than that in the dry treatment and that of the other species (Table 3).

G/R of *S. mexicanum* declined significantly with decreased frequency of irrigation (Table 3). In contrast, *S. aizoon* showed no difference in G/R between dry and nonirrigation treatments. However, all species and treatments had a G/R greater than 1.0, which indicates all plants have not withered and are still green. Moreover, although *Z. matrella* and *O. japonicus* in the wet

treatments were watered every day and not analyzed soil water content, this result suggests that plants in the wet treatment did not experience drought or waterlogging stress, which greatly affect plant growth.

MORPHOLOGICAL RESPONSES TO WATER REGIMES OF TWO *SEDUM* SPECIES AND COMPARISON WITH *Z. MATRELLA* AND *O. JAPONICUS*. The morphological responses of all four species at 28, 35, 42, and 49 d are presented in Table 4. All morphological traits of *S. mexicanum* were significantly affected by the difference in frequency of irrigation. LMF, SMF, and SLA in the wet treatment were significantly higher than those in the nonirrigation treatment, and RMF

in the wet treatment was significantly lower than that in dry and nonirrigation treatments. The morphological response of *S. aizoon* was similar to that of *S. mexicanum*, but there was no significant difference in treatments on SLA. The only species that flowered during the experiment was *S. aizoon* (wet treatment: flowering rate = 15/15, FMF = 0.14 ± 0.02; dry treatment: flowering rate = 5/15, FMF = 0.013 ± 0.006). It is well documented that plants respond to a decrease in below-ground resources (e.g., water, nutrients) with increased biomass allocation to roots (Poorter and Nagel, 2000) which is consistent with the results of LMF, SMF, and RMF in this study.

LMF of the two *Sedum* species in wet and dry treatments was significantly higher than that of *Z. matrella* and *O. japonicas* (Table 4). The SMF and RMF results clearly demonstrate that *Z. matrella* allocated significantly more biomass to stems, and *O. japonicas* allocated significantly more biomass to roots. SLA of the *Z. matrella* and *O. japonicus* exhibited similar values to that of *S. mexicanum* in the nonirrigation treatment, and SLA of *S. mexicanum* in wet and dry treatments were significantly higher than that of the other species.

In green roofs, LAI and SLA were evaluated as predictors of environmental benefits (Lundholm et al., 2015; Takakura et al., 2000). There may be differences in growth condition and stage between this experiment and actual green roofs. However, results of the current study clearly indicate that these morphological indices of the two *Sedum* species significantly change with changes in below-ground resources (water and nutrients), which suggest that appropriate water and nutrient management improves the environmental benefits of *Sedum* green roofs. In

addition, as shown by the differing changes in SLA of the two *Sedum* species, species have individual traits and respond differently to below-ground resources. From the standpoint of morphological traits of leaves, *S. mexicanum* may be more appropriate for a green roof based on this study. Thus, it is necessary to consider the morphological traits of the plants involved to design a more effective green roof.

QUANTITATIVE ANALYSIS OF CARBON SEQUESTRATION AND THE INFLUENCE OF PHYSIOLOGICAL AND MORPHOLOGICAL TRAITS USING GROWTH ANALYSIS. Carbon concentration of the two *Sedum* species did not differ significantly between treatments, but there were significant differences between species (Table 5). Initial carbon content did not differ significantly between *S. mexicanum* (0.20 ± 0.01 g), *S. aizoon* (0.21 ± 0.01 g), and *Z. matrella* (0.21 ± 0.02 g), but *O. japonicus* (0.27 ± 0.01 g) had greater carbon content (Dunnnett-T3 at $P < 0.05$). Carbon sequestration of the *Sedum* species was significantly influenced by the frequency of irrigation (Table 5). At the end of the experiment, carbon content of *Z. matrella* was similar to that of *Sedum* in the wet treatments, whereas *O. japonicus* was similar to that of *Sedum* in the dry treatments. The time course of whole-plant carbon content is shown in Fig. 4. Average carbon content increased across the study period.

Results of the growth analysis are shown in Table 6. RGR_c of the two *Sedum* species was clearly affected by the difference in frequency of irrigation and was similar in each treatment. NAR_c and LAR_c of the two *Sedum* species were also influenced by the difference in frequency of irrigation, except for the LAR_c of *S. aizoon*.

In the wet treatment, *S. mexicanum* and *S. aizoon* had the highest RGR_c of the four species. *Sedum mexicanum* had the lowest NAR_c of the four species, but its LAR_c was more than double that of the others. *Sedum aizoon* had the second highest NAR_c and LAR_c in the wet treatment. In comparison, although *Z. materella* exhibited the highest NAR_c , its RGR_c was lower than that of the two *Sedum* species because its LAR_c was lower than that of *Sedum* in all treatments. *Ophiopogon japonicus* also had high NAR_c and the lowest LAR_c in this experiment, which resulted in RGR_c being lower than that of *Sedum* in the dry treatment. The four plant species in the wet treatment had a significant correlation ($r = 0.972$, $P < 0.05$) between NAR_c and photosynthetic rate (25°C , $1000\ \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). In addition, LAR is commonly known as an index derived from LMF and SLA in growth analyses. There was significant correlation ($r = 0.959$, $P < 0.01$) between LAR_c and the product of LMF and SLA in all species and treatments.

GRC_{NAR_c} and GRC_{LAR_c} of *S. mexicanum* were about 0.7 and 0.3, respectively, in all comparisons (Table 7). This result indicates that 70% of a change in RGR_c (competence for carbon sequestration) was due to changes in NAR_c and 30% was due to changes in LAR_c . In contrast, GRC_{NAR_c} of *S. aizoon* was about 0.95, so competence for carbon sequestration was strongly affected by a change in NAR_c . *Sedum aizoon* did not differ in

Table 4. Mean values ($n = 15$) for biomass allocation as measured by leaf mass fraction (LMF), stem mass fraction (SMF), root mass fraction (RMF), and specific leaf area (SLA) of two *Sedum* species (*Sedum mexicanum* and *Sedum aizoon*) in wet treatment (plants supplied nutrient solution every day), dry treatment (plants supplied nutrient solution once per week), and nonirrigation treatment [plants only supplied nutrient solution at the start of the experiment (0 d)] at the 28, 35, 42, and 49 d after the start of experiment, and those of two green roof plants (*Zoysia matrella* and *Ophiopogon japonicus*) in the wet treatment.

Species	Treatment	LMF	SMF	RMF	SLA
		(mean $\pm$ SE)	(mean $\pm$ SE)	(mean $\pm$ SE)	[mean $\pm$ SE ($\text{m}^2\cdot\text{g}^{-1}$)]
<i>S. mexicanum</i>	Wet	0.61 ± 0.00 d ^z	0.30 ± 0.01 e ^z	0.09 ± 0.01 a ^z	0.046 ± 0.002 d ^z
	Dry	0.52 ± 0.01 cd	0.23 ± 0.01 cd	0.25 ± 0.01 e	0.039 ± 0.002 d
	Nonirrigation	0.46 ± 0.02 bc	0.20 ± 0.01 bc	0.34 ± 0.02 de	0.021 ± 0.001 bc
<i>S. aizoon</i>	Wet	0.58 ± 0.02 d	0.20 ± 0.01 b	0.09 ± 0.01 a	0.017 ± 0.001 ab
	Dry	0.60 ± 0.01 d	0.12 ± 0.00 a	0.27 ± 0.01 cd	0.016 ± 0.000 a
	Nonirrigation	0.48 ± 0.01 c	0.11 ± 0.00 a	0.41 ± 0.01 e	0.016 ± 0.000 a
<i>Z. matrella</i>	Wet	0.40 ± 0.01 bc	0.47 ± 0.01 f	0.13 ± 0.01 b	0.024 ± 0.001 c
<i>O. japonicus</i>	Wet	0.33 ± 0.01 a	0.25 ± 0.01 d	0.41 ± 0.01 e	0.020 ± 0.001 b

^zSignificant differences among means are indicated by different letters based on Dunnnett-T3 at $P < 0.05$.

Table 5. Mean values ($n = 6$) for carbon concentration and carbon content of two *Sedum* species (*Sedum mexicanum* and *Sedum aizoon*) in wet treatment (plants supplied nutrient solution every day), dry treatment (plants supplied nutrient solution once per week), and nonirrigation treatment [plants only supplied nutrient solution at the start of the experiment (0 d)] at the end of the experiment (49 d), and those of two green roof plants (*Zoysia matrella* and *Ophiopogon japonicus*) in the wet treatment.

Species	Treatment	C	Carbon content
		[mean $\pm$ SE (%)]	[mean $\pm$ SE (g/plant)]
<i>S. mexicanum</i>	Wet	37.4 ± 0.4 ab ^z	3.4 ± 0.2 C ^z
	Dry	37.4 ± 0.3 a	1.1 ± 0.0 B
	Nonirrigation	37.9 ± 0.5 abc	0.3 ± 0.0 A
<i>S. aizoon</i>	Wet	38.8 ± 0.3 bc	3.7 ± 0.2 c ^z
	Dry	39.7 ± 0.2 cd	0.9 ± 0.0 b ^z
	Nonirrigation	38.8 ± 0.4 abcd	0.3 ± 0.0 a ^z
<i>Z. matrella</i>	Wet	42.6 ± 0.1 e	3.2 ± 0.2
<i>O. japonicus</i>	Wet	40.3 ± 0.2 d	0.8 ± 0.1

^zSignificant differences among means are indicated by different letters based on Dunnnett-T3 at $P < 0.05$.

SLA among treatments, which may be related to this result (Table 4). Thus, variation in carbon sequestration for different below-ground resources seemed to be mainly caused by physiological responses.

The NAR_c and LAR_c results were consistent with the results for photosynthetic rates and biomass allocation. This finding suggested that growth analysis reveals the relevance of physiological (NAR_c) and morphological (LAR_c) traits and competence for carbon sequestration (RGR_c). Thus, it became clear that the appropriate water and nutrient regime could elevate the competence for carbon sequestration in a *Sedum* green roof as a result of physiological and morphological responses.

Moreover, there is an suggestion that *Sedum* has higher competence for carbon sequestration than a C_4 plant (*Z. matrella*) in appropriate below-ground resource (water and nutrient) conditions for *Sedum* species, and even a *Sedum* green roof with a low frequency of irrigation or rain events may show higher carbon sequestration than another green roof plant (*O. japonicus*).

Fig. 4. Time course of mean values for carbon content of two *Sedum* species [(A) *Sedum mexicanum*, (B) *Sedum aizoon*] in wet treatment (plants supplied nutrient solution every day), dry treatment (plants supplied nutrient solution once per week), and nonirrigation treatment (plants only supplied nutrient solution at the start of the experiment [0 d]), and those of two other green plants [(C) *Zoysia matrella* and *Ophiopogon japonicus*] in wet treatment. Means $\pm$ SE are presented ($n = 3$, except for after 3 d and after 49 d where $n = 6$).

From the standpoint of application to actual green roofs, this study did not cover all green roof conditions (e.g., wind speed, high and low temperatures, light intensities, and nutrient conditions) which may have led to different growth of plants as compared with those in actual extensive green roofs. Thus, for other environmental conditions and plant growth stages, the relevance of physiological and morphological traits and environmental benefits should be studied further. Our results will serve as a baseline of the physiological and morphological traits and

Table 6. Relative C sequestration rate per whole-plant C content (RGR_c), net assimilation rate (NAR_c), and leaf area ratio per whole-plant C content (LAR_c) of two *Sedum* species (*Sedum mexicanum* and *Sedum aizoon*) in wet treatment (plants supplied nutrient solution every day), dry treatment (plants supplied nutrient solution once per week), and nonirrigation treatment [plants only supplied nutrient solution at the start of the experiment (0 d)] and those of two green roof plants (*Zoysia matrella* and *Ophiopogon japonicus*) in the wet treatment.

Species	Treatment	RGR_c (per d)	NAR_c ($g \cdot m^{-2} \cdot d^{-1} C$)	LAR_c ($m^2 \cdot g^{-1} C$)
<i>S. mexicanum</i>	Wet	0.062	1.07	0.059
	Dry	0.035	0.73	0.048
	Nonirrigation	0.008	0.26	0.031
<i>S. aizoon</i>	Wet	0.062	2.52	0.024
	Dry	0.031	1.26	0.024
	Nonirrigation	0.006	0.29	0.022
<i>Z. matrella</i>	Wet	0.053	3.34	0.017
<i>O. japonicus</i>	Wet	0.019	1.34	0.014

Table 7. Growth response coefficients (GRC) of net assimilation rate (NAR_c) and leaf area ratio per whole-plant C content (LAR_c) in two *Sedum* species (*Sedum mexicanum* and *Sedum aizoon*) between wet and dry, wet and nonirrigation, and dry and nonirrigation treatments [wet treatment: plants supplied nutrient solution every day, dry treatment: plants supplied nutrient solution once per week, nonirrigation treatment: plants only supplied nutrient solution at the start of the experiment (0 d)].

Species	Comparison	GRC_{NAR_c}	GRC_{LAR_c}
<i>S. mexicanum</i>	Wet and dry	0.68	0.36
	Wet and nonirrigation	0.69	0.31
	Dry and nonirrigation	0.69	0.29
<i>S. aizoon</i>	Wet and dry	0.99	0.01
	Wet and nonirrigation	0.94	0.05
	Dry and nonirrigation	0.92	0.07

carbon sequestration of green roof plants, and contribute to more suitable design and maintenance of vegetation in green roofs.

Conclusions

This study compared physiological and morphological traits of four green roof plants and discussed the influence of these traits on their environmental benefits in controlled conditions. In wet and increased nutrient conditions, *S. mexicanum* and *S. aizoon* exhibited the C_3 photosynthetic pathway, and their LMF significantly increased in comparison with the dry and non-irrigation treatments. Consequently, transpiration rates and competence for carbon sequestration (RGR_c) of the two *Sedum* species were higher than those of *Z. matrella* and *O. japonicus* in our experiment.

Although this study do not cover all green roof conditions and plant growth stages, our results serve as a baseline of the physiological and morphological traits and carbon sequestration of green roof plants. In addition, growth analysis performed on plant carbon content enabled the relevance of physiological (NAR_c) and morphological (LAR_c) traits and competence for carbon sequestration (RGR_c) in green roof plants to be understood more clearly. This analysis method could apply to other experiments and green plants. Our results

and analysis method will contribute to more suitable design and maintenance of vegetation in green roofs.

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