Ecophysiological Differentiation of Two *Mosla* Species in Response to Nitrogen and Water Levels

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Terrestrial ecosystems are experiencing increased inputs of nitrogen (N) and temporal fluctuations in precipitation, causing flooding or drought, and this could strongly affect the fate of terrestrial plant species, as they might have different abilities to adapt to the changing environment. We grew *Mosla dianthera* (a widespread species) and *M. hangchowensis* (an endangered species) under three water treatments (drought, sufficient water, and waterlogging) in combination with three levels of N supply (low, intermediate, and sufficient N) to study the ecophysiological responses of the congeneric species to those simulated environmental changes. The two species showed different responses to waterlogging and drought treatments, particularly when there was abundant N supply in the system. For example, under sufficient N but drought or waterlogging conditions, *M. dianthera* increased root mass ratio (RMR) and decreased leaf mass ratio (LMR), total leaf area (LA), and leaf area ratio (LAR); such changes can enhance water acquisition and reduce water loss under both drought and waterlogging conditions, in contrast to the general lack of change in those parameters with *M. hangchowensis*. These differentiations in traits suggest that increased N availability might worsen drought and waterlogging injury to *M. hangchowensis* and thus accelerate the decline of this population. However, *M. dianthera* maybe better adapted to high N availability and both drought and waterlogging conditions. We hypothesize that the different adaptive abilities to high N availability and drought and waterlogging conditions are partly responsible for the ecological differentiation observed between these two species in the field and may determine their fate in their native habitat. Further research should test this hypothesis in field experiments.

Keywords Deficiency, drought, endangered species, nitrogen, waterlogging

Introduction

Terrestrial ecosystems are experiencing increased inputs of nitrogen (N) through atmospheric deposition (Clark and Tilman 2008) and large spatial and temporal heterogeneity

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in precipitation (Folland et al. 2001). The latter will likely increase frequencies of flooding and drought events (Qian, Fu, and Yan 2007). Such changes will have both direct and indirect effects on soil N availabilities. For example, an increased rate of N deposition will directly increase N availability in the soil (Penn et al. 2003). Increased precipitation can increase N leaching and surface runoff and lead to N shortage for plant growth, especially on sloping highland regions (Kawakami, Honoki, and Yasuda 2001), while N availabilities will increase in lowland areas due to N flowing from higher grounds (Whitehead, Hill, and Neal 2004).

Available N and water contents in the soil play fundamental roles in determining the growth, abundance, and distribution of most plant species (Schulze et al. 1987; Hobbs and Mooney 1991; Abreu et al. 1993; Matsushima and Chang 2007; Tilman 1988). Long-term environmental stresses can lead to natural selection on specific morphological and physiological traits to ultimately cause ecological differentiation (Schluter 2001) and determine the species’ future fate in relation to global environment changes. Therefore, studying the ecophysiological responses of plant species of closely related taxa to varied N and water stresses will help us better understand the ecological impact of global environmental changes.

In the genus *Mosla* Buch.-Ham. ex Maxim. (Lamiaceae), there are only eight species that are distributed in East and Southeast Asia (mainly in South and East China) (Zhou 1995). Among those species, *M. dianthera* (Buch.-Ham.) Maxim. is widely distributed and is often a dominant species in the communities. Its habitats range from shedding hills to the edge of wetlands with alkali-hydrolyzable N concentrations in soils range from 56 ± 3.4 (mean ± SE) to 269 ± 28.7 mg N kg\(^{-1}\) (Jie Chang, unpublished data). The congeneric species *M. hangchowensis* Matsuda is now considered endangered and has only three small local populations in the Yangtze River Delta region, where its distribution overlaps with *M. dianthera* (Fang et al. 1989; Liao et al. 2006). The habitats of *M. hangchowensis* are limited to rocky hilltops and/or gravel roadsides in high mountain regions with thin top soil (Chang et al. 1999; Ge and Chang 2001) with high soil alkali-hydrolyzable N concentrations (range from 558 ± 13.2 to 757 ± 2.3 mg N kg\(^{-1}\)) (Jie Chang, unpublished data). In the past 50 years, *M. dianthera* populations remained stable while *M. hangchowensis* populations had been reduced from eight to three (Ge and Chang 2001; Liao et al. 2006). See Figure 1 for their current distribution.

The region where *M. dianthera* and *M. hangchowensis* coexist is densely populated and is being increasingly affected by atmospheric N deposition (Lü and Tian 2007), where N deposition rates can be as high as 38 kg N ha\(^{-1}\) yr\(^{-1}\) (Gu et al. 2009). The region has a mean annual precipitation of 1,400 mm, and extreme weather events are becoming more common (NBSC 2001–2007). While the exact mechanism for decline of the *M. hangchowensis* population is unclear, we hypothesize that variations in soil N availabilities and water status cause differential ecophysiological responses of *M. dianthera* and *M. hangchowensis*, and such differentiation could be one of the reasons for the reduction of populations of *M. hangchowensis* but not of *M. dianthera*. A greenhouse experiment was conducted to investigate the growth, biomass allocation, and physiological responses of these two species under different N and water treatments. The objective of this study was to examine the ecophysiological responses of *M. dianthera* and *M. hangchowensis* to variations in soil N and water availabilities.
Responses of Mosla Species to N and Water Levels

Figure 1. Progressive reduction of the distribution of *M. hangchowensis* populations: from the 1950s (dashed ellipse) to the 1990s (solid ellipse) and 2000s (dotted ellipse).

### Materials and Methods

**Plant Materials**

Seedlings of *M. dianthera* and *M. hangchowensis* were collected from Geling (120° 08′ E, 30° 15′ N) and Wuchao Mountains (120° 00′ E, 30° 12′ N), respectively, in April 2006. The seedlings were grown at Hangzhou Botanical Garden (120° 16′ E, 30° 15′ N) under natural conditions for seed harvest. Seeds were then air dried and stored in a refrigerator (4 °C) (SC-329GA, Haier Group, China) in November 2006.

Seeds were germinated in mid-March 2007 in trays of peat (Sunshine Mix 6, Sun Gro Horticulture Canada Ltd., Canada) in a growth chamber with a 16-h photoperiod, day/night temperatures of 25/15 °C, and 70–80% relative humidity and irradiance (λ = 400–700 nm) of c. 250 μmol photon m⁻² s⁻¹. After germination, the seedlings were watered with a full-strength modified Hoagland’s nutrient solution (Table 1). When the seedlings reached about 5 cm in height, they were transplanted to pots (12 cm in diameter and 9 cm deep) filled with 120 g commercial peat, vermiculite, and perlite mixture at a volume ratio of 10:7:3 in April 2007. Plants were grown for 10 weeks in a greenhouse on the campus of Zhejiang University (120° 05′ E, 30° 18′ N), Hangzhou, with natural irradiation conditions.
Table 1
Amount of chemicals (mg of chemical L\(^{-1}\)) used to make up the modified Hoagland’s nutrient solution used for the three N levels

<table>
<thead>
<tr>
<th>Major elements</th>
<th>LN</th>
<th>MN</th>
<th>SN</th>
</tr>
</thead>
<tbody>
<tr>
<td>KNO(_3)</td>
<td>60.7</td>
<td>242.6</td>
<td>606.6</td>
</tr>
</tbody>
</table>
| Ca(NO\(_3\))\(_2\)
·4H\(_2\)O       | 94.5   | 377.9  | 944.6  |
| CaCl\(_2\)
·2H\(_2\)O       | 529.3  | 352.9  | 0      |
| NH\(_4\)H\(_2\)PO\(_4\) | 23.0   | 92.1   | 230.2  |
| KH\(_2\)PO\(_4\) | 244.8  | 163.2  | 0      |
| MgSO\(_4\)
·7H\(_2\)O       | 246.5  | 246.5  | 246.5  |
| KCl             | 268.4  | 178.9  | 0      |

\(a\) The three N levels contain the same concentrations of micronutrients (mg of chemical L\(^{-1}\)): H\(_3\)BO\(_3\) = 1.55, MnSO\(_4\)
·7H\(_2\)O = 0.34, ZnSO\(_4\)
·7H\(_2\)O = 0.58, CuSO\(_4\)
·5H\(_2\)O = 0.12, H\(_2\)MoO\(_4\) (85% MoO\(_3\)) = 0.08, and NaFeDTPA (10% Fe) = 15.00. LN, MN, and SN refer to 10, 40, and 100% N concentrations of full-strength modified Hoagland’s nutrient solution (Hoagland and Arnon 1950; Wassner and Ravetta 2007).

The average daily temperature ranged from 20 to 36 °C during the experiment, which lasted from April to July 2007.

**Experimental Design**

The experiment involved three factors (N addition, water level, and species) in a \(3 \times 3 \times 2\) completely randomized factorial design with six replications per treatment, with a total of 108 pots. The first factor included three levels of N concentrations (Table 1): 10% (22.4 mg N L\(^{-1}\)), 40% (89.6 mg N L\(^{-1}\)), and 100% (224 mg N L\(^{-1}\)) N concentration of full-strength modified Hoagland’s nutrient solution with addition of calcium chloride (CaCl\(_2\)), monopotassium phosphate (KH\(_2\)PO\(_4\)), and potassium chloride (KCl) to maintain equal calcium (Ca\(^{2+}\)), potassium (K\(^{+}\)), and dihydrogen phosphate (H\(_2\)PO\(_4^{-}\)) concentrations in each treatment. Those treatments are referred to as low N (LN), intermediate N (MN), and sufficient N (SN), respectively. Nutrient solutions were added evenly every week at a rate of 15 mL per pot. The second factor included three levels of watering regime to maintain soil moisture content at 30% and 90% soil water-holding capacity (WHC) and waterlogged. Those treatments are referred to as low water (LW), sufficient water (SW), and waterlogged (WL), respectively. Under WL, the pots were placed in plastic bags and maintained with c. 20 mm of water above the soil surface. We weighed pots under the LW and SW treatments periodically to determine when the pots need to be watered. The pots were rotated regularly to avoid confounding effects of light and temperature gradients within the greenhouse.

**Measurements and Calculation**

On 30 June 2007, when the plants reached peak growth, the height of all plants was measured. Then, leaf net photosynthetic rate (\(P_N\)) and dark respiration (\(R\)) were measured on two mature leaves near the top of the plant using a portable gas-exchange system (model LCA-4, ADC, Hoddesdon, UK). The \(P_N\) was measured between 9:30 and 12:00, and \(R\) was measured between 19:00 and 23:00. Photosynthetically active radiation (PAR) of 1000
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μmol photon m\(^{-2}\) s\(^{-1}\) was supplied by a halogen lamp fitted with an infrared absorbing filter (portable light unit type PLU-LMC-002, ADC, Hoddesdon, UK) for the measurement of \(P_N\). Prior to conducting the \(R\) measurement, plants were kept in a dark growth chamber for 30 min. The carbon dioxide (CO\(_2\)) concentration and temperature in the leaf chamber were maintained at 380 \(\mu\)mol mol\(^{-1}\) and 25 °C, respectively. The \(P_N\) and \(R\) (μmol m\(^{-2}\) s\(^{-1}\)) values were expressed on a unit leaf area basis.

After the gas-exchange measurement, the plants were harvested and separated into above- and belowground parts. The aboveground plant was further separated into stems and leaves. All leaves of each plant were scanned with a scanner (ScanMaker 4900, Microtek International Inc., USA) immediately after harvesting to determine the total leaf area using the WinFLORA Pro 2002a (Regent Instruments Inc., Quebec, Canada) software. The root system was placed over a 2-mm screen and washed with a gentle stream of water.

After all these measurements, all samples were dried in an oven at 65 °C for at least 48 h to constant weight to determine biomass. Specific leaf area (SLA, leaf area / leaf mass), leaf area ratio (LAR, leaf area / total mass), leaf mass ratio (LMR, leaf mass / total mass), stem mass ratio (SMR, stem mass / total mass), root mass ratio (RMR, root mass / total mass), and root-to-shoot ratio (R/S, root mass / aboveground mass) were calculated following Hunt (1978) and Sakai (1995).

Statistical Analysis

Normality of the dataset was tested by the Shapiro-Wilks test using the SPSS 15.0 for Windows software package (SPSS Inc., Chicago, Ill.). Data for total biomass of \(M.\) dianthera and \(M.\) hangchowensis and R/S of \(M.\) dianthera were square-root transformed, and total leaf area of \(M.\) hangchowensis was logarithmically transformed before analysis to correct the heterogeneity of variances. The untransformed data are presented in the tables and figures. Data were analyzed using a two-way analysis of variance (ANOVA) for the factors of N and watering levels for each species, using the EMMEANS in General Linear Model (GLM) procedure of SPSS (SPSS Inc., Chicago, Ill.). The overall interspecific differences of the two Mosla species in their responses to various N and water treatments were evaluated using principal component analyses (PCA) using Statistica version 6.0 (StatSoft Inc., Okla.). Statistical significance was set at \(\alpha = 0.05\) unless otherwise noted.

Results

Total Biomass

For both species, total biomass increased with increasing N level (\(P < 0.05\), ANOVA data are in Table 2; Figures 2a and 2b). For \(M.\) dianthera, under LN condition, total biomass was less under LW and WL compared with SW; whereas under MN and SN, total biomass was the least under LW (\(P < 0.01\)). However, unlike \(M.\) dianthera, the total biomass of \(M.\) hangchowensis was affected by both N and water levels, but there was no N × water interaction (Figure 2 and Table 2).

Biomass Allocation

For \(M.\) dianthera, RMR was greater under LW and WL than under SW regardless of the N level, but RMR was not affected by N levels under LW (Figure 3a). Under SW and WL, RMR was the greatest under LN. In contrast, RMR of \(M.\) hangchowensis was the greatest under LN independent of the water level (\(P < 0.001\), Figure 3b) and was greater under LW than under SW or WL when grown under LN or MN. Compared with SW, WL decreased
Table 2

<table>
<thead>
<tr>
<th>Parameters</th>
<th>M. dianthera</th>
<th>M. hangchowensis</th>
</tr>
</thead>
<tbody>
<tr>
<td>$M_T$</td>
<td>65.1***</td>
<td>15.1***</td>
</tr>
<tr>
<td>RMR</td>
<td>3.6*</td>
<td>19.9***</td>
</tr>
<tr>
<td>LMR</td>
<td>11.3***</td>
<td>10.1***</td>
</tr>
<tr>
<td>SMR</td>
<td>27.0***</td>
<td>11.2***</td>
</tr>
<tr>
<td>Height</td>
<td>101.7***</td>
<td>35.6***</td>
</tr>
<tr>
<td>LA</td>
<td>22.9***</td>
<td>127.6***</td>
</tr>
<tr>
<td>LAR</td>
<td>30.3***</td>
<td>21.9***</td>
</tr>
<tr>
<td>$P_N$</td>
<td>43.4***</td>
<td>55.5***</td>
</tr>
<tr>
<td>$R$</td>
<td>26.3***</td>
<td>25.4***</td>
</tr>
<tr>
<td>$R/P$</td>
<td>61.8***</td>
<td>36.9***</td>
</tr>
</tbody>
</table>

$aM_T$, total biomass; RMR, root mass ratio; LMR, leaf mass ratio; SMR, stem mass ratio; LA, total leaf area; LAR, leaf area ratio; $P_N$, net photosynthetic rate; $R$, dark respiration; and $R/P$, dark respiration to net photosynthesis ratio.

*P < 0.05, **P < 0.01, ***P < 0.001.

![Figure 2](image-url)
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Figure 3. Root mass ratio (RMR), leaf mass ratio (LMR), and stem mass ratio (SMR) of M. dianthera and M. hangchowensis grown under three N levels in combination with three water levels. LN, MN, and SN refer to 10, 40, and 100% N concentrations of full-strength modified Hoagland’s nutrient solution, respectively. LW, SW, and WL refer to 30 and 90% soil water-holding capacity and waterlogging, respectively. Error bars are standard errors of means.

N level ($P < 0.001$). For M. hangchowensis, under LN and MN conditions, the LW and WL treatments reduced LMR as compared with SW, whereas no difference was observed among the three water levels under the SN condition (Figure 3d). For both species, SMR increased with increasing N levels regardless of the water level ($P < 0.001$), and it was the greatest under WL regardless of the N level ($P < 0.001$; Figures 3e and 3f).
**Morphological Features**

Height of both species increased with increasing N and water levels ($P < 0.001$; Figures 4a, 4b). Total leaf area of *M. dianthera* was less under LN than under MN and SN, independent of water level (Figure 4c). In response to water levels, total leaf area of *M. dianthera* was the greatest under SW, independent of N level ($P < 0.001$). However, total leaf area of

![Figure 4](image-url)

**Figure 4.** Height, total leaf area, and leaf area ratio (LAR) of *M. dianthera* and *M. hangchowensis* grown under three N levels in combination with three water levels. LN, MN, and SN refer to 10, 40, and 100% N concentrations of full-strength modified Hoagland’s nutrient solution, respectively. LW, SW, and WL refer to 30 and 90% soil water-holding capacity and waterlogging, respectively. Error bars are standard errors of means.
M. hangchowensis increased with increasing N levels regardless of the water treatment (P < 0.001; Figure 4d). In response to water levels, under LN and MN, LW rather than WL reduced total leaf area as compared with SW (P < 0.001), and there was no difference among three water levels under SN.

The SLA was not affected by N or water levels for both species (data not shown). For M. dianthera, LAR was the greatest under SW independent of N level (Figure 4e). For M. hangchowensis, both LW and WL resulted in reductions of LAR as compared with the SW treatment when the N treatment is either LN or MN (Figure 4f). However, under SN, water level did not affect LAR.

**Table 3**
Net photosynthetic rate ($P_N$), dark respiration ($R$), and $R$ to $P_N$ ratio ($R/P$) of M. dianthera and M. hangchowensis grown under a combination of three N and three water levels

<table>
<thead>
<tr>
<th>Species</th>
<th>Water treatment</th>
<th>Nitrogen treatment $^b$ ($\mu$mol m$^{-2}$ s$^{-1}$)</th>
<th>$P_N$ ($\mu$mol m$^{-2}$ s$^{-1}$)</th>
<th>$R$ ($\mu$mol m$^{-2}$ s$^{-1}$)</th>
<th>$R/P$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>M. dianthera</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LW</td>
<td>LN</td>
<td>3.95 ± 0.43$^{bc}$</td>
<td>2.89 ± 0.02$^{aA}$</td>
<td>0.75 ± 0.08$^{aA}$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MN</td>
<td>5.84 ± 0.33$^b$</td>
<td>2.11 ± 0.15$^{bB}$</td>
<td>0.36 ± 0.01$^{bA}$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SN</td>
<td>6.94 ± 0.58$^a$</td>
<td>1.59 ± 0.12$^{cB}$</td>
<td>0.24 ± 0.04$^{cA}$</td>
<td></td>
</tr>
<tr>
<td>SW</td>
<td>LN</td>
<td>6.91 ± 0.68$^A$</td>
<td>2.56 ± 0.02$^{aA}$</td>
<td>0.38 ± 0.05$^{abB}$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MN</td>
<td>9.67 ± 0.31</td>
<td>1.33 ± 0.07$^{bcC}$</td>
<td>0.14 ± 0.01$^{bB}$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SN</td>
<td>12.17 ± 0.55</td>
<td>1.47 ± 0.01$^{bB}$</td>
<td>0.12 ± 0.01$^{bB}$</td>
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<tr>
<td>WL</td>
<td>LN</td>
<td>7.67 ± 0.44$^A$</td>
<td>2.60 ± 0.04$^{aA}$</td>
<td>0.34 ± 0.02$^{abB}$</td>
<td></td>
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<tr>
<td></td>
<td>MN</td>
<td>9.89 ± 0.80</td>
<td>2.68 ± 0.34$^{abA}$</td>
<td>0.27 ± 0.01$^{abB}$</td>
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</tr>
<tr>
<td></td>
<td>SN</td>
<td>12.30 ± 0.73</td>
<td>2.58 ± 0.15$^{aA}$</td>
<td>0.21 ± 0.02$^{bAB}$</td>
<td></td>
</tr>
<tr>
<td><strong>M. hangchowensis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LW</td>
<td>LN</td>
<td>8.61 ± 0.39$^{abB}$</td>
<td>5.39 ± 0.43$^{abB}$</td>
<td>0.63 ± 0.06$^{abA}$</td>
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</tr>
<tr>
<td></td>
<td>MN</td>
<td>8.31 ± 0.76$^{abA}$</td>
<td>5.20 ± 0.63$^{a}$</td>
<td>0.62 ± 0.02$^{a}$</td>
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<tr>
<td></td>
<td>SN</td>
<td>8.99 ± 0.16$^{AB}$</td>
<td>5.74 ± 0.11$^{a}$</td>
<td>0.64 ± 0.01$^{a}$</td>
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</tr>
<tr>
<td>SW</td>
<td>LN</td>
<td>13.14 ± 0.65$^{bA}$</td>
<td>4.39 ± 0.06$^{B}$</td>
<td>0.34 ± 0.01$^{B}$</td>
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<tr>
<td></td>
<td>MN</td>
<td>15.73 ± 0.35$^{abA}$</td>
<td>5.23 ± 0.08</td>
<td>0.33 ± 0.01</td>
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<tr>
<td></td>
<td>SN</td>
<td>16.88 ± 0.62$^{abA}$</td>
<td>5.64 ± 0.04</td>
<td>0.33 ± 0.01</td>
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<tr>
<td>WL</td>
<td>LN</td>
<td>13.71 ± 0.46$^{bA}$</td>
<td>5.62 ± 0.66$^{A}$</td>
<td>0.41 ± 0.05$^{B}$</td>
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<tr>
<td></td>
<td>MN</td>
<td>15.45 ± 0.72$^{abA}$</td>
<td>6.45 ± 0.43</td>
<td>0.42 ± 0.01</td>
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<tr>
<td></td>
<td>SN</td>
<td>16.21 ± 0.86$^{abA}$</td>
<td>6.63 ± 0.09</td>
<td>0.41 ± 0.03</td>
<td></td>
</tr>
</tbody>
</table>

$^a$LW, SW, and WL refer to 30 and 90% soil water-holding capacity and waterlogging, respectively. $^b$LN, MN, and SN refer to 10, 40, and 100% N concentration of full-strength modified Hoagland's nutrient solution, respectively. Different lowercase letters indicate significant differences among N levels under the same water level; different capital letters indicate significant differences among water levels under the same N level. Mean separations that appear only for one set of data indicates nonsignificant N × water interactions. Data are presented as mean ± standard error, and statistical significance was set at $\alpha = 0.05$. 

**PN, R, and R-to-PN ratio (R/P)**

For M. dianthera, $P_N$ increased with increasing soil N levels regardless of the water level ($P < 0.001$; Tables 2 and 3). In response to water levels, only LW reduced $P_N$ as compared with SW and WL, independent of the N level ($P < 0.001$). Dark respiration ($R$) was greater
under LN than under MN or SN when the water treatment was either LW or SW; $R$ was not affected by the N treatment when the water treatment was WL. In comparison, under LN the water treatment did not affect $R$, whereas under MN and SN treatments $R$ was reduced under SW and LW as compared with WL. The $R/P$ was the greatest under LN when the water level was LW or SW (Table 3), and it was also the greatest under LW when the N level was either LN or SN.

For *M. hangchowensis* grown under LW, $P_N$ showed no difference among the three N levels (Tables 2 and 3). Under SW and WL, both MN and SN increased $P_N$ as compared with LN treatment. In response to water levels, only LW reduced $P_N$ as compared with SW and WL ($P < 0.001$). For this species, neither $R$ nor $R/P$ were affected by the N treatment (Table 3). For $R$, it was smaller under SW than under WL regardless of the N treatment. The LW reduced $R/P$ as compared with SW and WL regardless of the N treatment (Table 3).

### Principal Component Analysis (PCA)

The PCA analysis resulted in eigenvalue of 0.466 for the first axis and 0.294 for the second axis (Figure 5). *Mosla hangchowensis* was relatively concentrated, whereas *M. dianthera* was rather scattered in the principal component plot. The results showed that the first axis was mainly explained by RMR and $R/S$, whereas the second axis was mainly explained by SMR and height. The two species were separated from each other mainly along the first axis, with *M. dianthera* characterized by greater RMR and $R/S$, and *M. hangchowensis* with lower RMR and $R/S$.

### Discussion

Oxygen deficiency is the primary limitation to plant growth and functioning under waterlogged conditions (Li, Pezeshki, and Goodwin 2004). Some hydrophytes are physiologically adapted to anoxic conditions. However, our results showed that the two *Mosla* species, as mesophytes, generally grew well for about 3 months under waterlogged conditions, as indicated by their height (Figures 4a and 4b) and biomass (Figures 2a and 2b). We observed that under WL conditions they produced aerial roots, which would help with oxygen acquisition (Larcher 1995). However, as compared with the SW treatment (which could be considered as a normal growth condition for mesophytes), *M. dianthera* under the WL treatment allocated relatively more biomass to stem and root (i.e., greater RMR and SMR values under WL than under SW conditions) by sacrificing its allocation to leaves (i.e., lower LMR values) as compared with *M. hangchowensis* (Figure 3). This change of allocation pattern is also reflected in the relative differences in total leaf area between SW and WL for the two species (Figures 4c and 4d). Allocating more biomass to stems at the expense of leaves to reduce the evaporative demand under the waterlogging condition is advantageous for adapting to such an adverse condition, as water uptake under waterlogging conditions is limited by low oxygen supply. The advantage of *M. dianthera* over *M. hangchowensis* against waterlogged conditions is also reflected in the increased $P_N$ (relative to the SW treatment) for the former as compared with no change for the latter (Table 3).

Similarly, increased belowground biomass allocation is also advantageous for plants growing in droughty or nutrient-limited conditions to maximize their ability to acquire water or nutrients (Gleeson and Tilman 1992). Lowering evaporative surface area is also an adaptive response of plants to drought (Schulze 1986). Similar to the differential responses
Figure 5. Principle component analysis for growth as well as morphological and physiological traits of *M. dianthera* (squares) and *M. hangchowensis* (triangles) in response to varied N and water levels. Each point in the upper graph denotes a mean value under a combination N-water level and the lower graph show factor loadings. Abbreviations are explained in the footnote of Table 2.
of the two species to waterlogging, *M. dianthera* showed an advantage over *M. hangchowensis* as well under the drought treatment in terms of reducing its allocation of biomass to leaf (relative to the SW treatment) but increasing its allocation of biomass to root and stem (Figure 3). Such an advantage is again corroborated by the smaller drop in \( P_N \) between SW and LW for *M. dianthera* than for *M. hangchowensis* (Table 3).

Therefore, for *M. dianthera*, the morphological and physiological adjustment to the water treatment was consistent regardless of the N level (Figures 3 and 4). In other words, *M. dianthera* readily adjusted its below- and aboveground biomass allocation and showed better adaptability to drought and waterlogging conditions. Although *M. hangchowensis* was generally less responsive to the water level treatments than *M. dianthera* in terms of their morphological and physiological adjustments, *M. hangchowensis* was least responsive to the water level treatment when there was sufficient N available in the soil (Figures 3 and 4). The failure to adjust morphologically and physiologically so that root and shoot systems can maintain the balance between water acquisition and consumption in a plant will increase the risk of injury under drought and waterlogging conditions for *M. hangchowensis*, particularly when the conditions in the field are changeable over the growing season and plants showing little ability to adapt could be poorly prepared for sudden changes in growth conditions.

Smaller plant size means lower consumption of resources for survival under adverse conditions (Larcher 1995). We found that total biomass of *M. dianthera* but not *M. hangchowensis* was reduced by drought and waterlogging as compared with sufficient water supply (Figure 2); we speculate that the smaller biomass may enable *M. dianthera* to be more tolerant to drought and waterlogging conditions than *M. hangchowensis*. This, however, needs to be tested under field conditions over multiple growing seasons. In addition, the two species showed some different responses to N levels in traits related to carbon production. For example, \( P_N \) of *M. dianthera* increased while \( R \) decreased with the increasing soil N levels, which led to a decreased \( R/P \); and the effects of N level on \( R \) and \( R/P \) were not significant for *M. hangchowensis* (Tables 2 and 3). Differences in the two species in their response to the N and water treatments are also strongly supported by the PCA (Figure 5).

The different patterns of morphological and physiological responses to soil N and water treatments indicate that such differences may lead to ecological differentiation between the two species as a result of long-term adaptation to different habitat conditions (Eriksen and Nordal 1989). Increased rates of N deposition in terrestrial ecosystems, especially in the Yangtze River Delta region (Lü and Tian 2007), will inevitably increase N availability in habitats of the two *Mosla* species. Unfortunately, frequencies of drought and waterlogging events in the natural habitats of these two species may increase (Qian, Fu, and Yan 2007). Our data here and past research (Chang et al. 1999; Ge and Chang 2001; Liao et al. 2006) suggest that *M. dianthera* has a greater ability to adjust its eco-physiological characteristics for survival and persistence in a broader range of N and water levels, whereas *M. hangchowensis* was less able to adapt to adverse conditions, which may be characterized by the excessive amount of available N and/or drought or waterlogging conditions. We hypothesize that the endangerment of *M. hangchowensis* and persistence of *M. dianthera* during the past 50 years might be partly related to N enrichment in its habitats as a result of N deposition. Because this study is a greenhouse experiment, this hypothesis needs to be tested under field conditions. If the hypothesis is accepted, we expect that *M. dianthera* will maintain dominance in the plant community while the distribution of *M. hangchowensis* will become further restricted in the face of environmental changes.
Responses of Mosla Species to N and Water Levels

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