Coal-Bed Methane Water Effects on Dill and Its Essential Oils

Shital Poudyal, Valtcho D. Zheljazkov,* Charles L. Cantrell, and Thijs Kelleners

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Pumping water from coal seams decreases the pressure in the seam and in turn releases trapped methane; this is the most common and economic method of methane extraction. The water that is pumped out is known as “coal-bed methane water” (CBMW), which is high in sodium and other salts. In the past 25 yr, the United States has seen a 16-fold increase in the production of coal bed methane gas, and trillions of cubic meters are yet to be extracted. There is no sustainable disposal method for CBMW, and there are very few studies investigating the effects of this water on plants and their secondary metabolites and on soil properties. This study was conducted to determine the effects of CBMW on soil chemical properties and on the biomass and essential oil yield and composition of dill (Anethum graveolens L.). This crop was grown in a greenhouse and was subjected to different levels of CBMW treatment: tap water only; 25% CBMW, 75% tap water; 50% CBMW, 50% tap water; 75% CBMW, 25% tap water; and 100% CBMW. The major dill oil constituents, limonene and α-phellandrene, were not affected by the treatments; however, the concentration of dill ether increased with increasing CBMW levels, whereas the concentration of carvone decreased. In soil, sodium level significantly increased with increasing level of treatment, but pH and cation exchange capacity were not much affected. Coal bed methane water could be used for irrigation of dill for one growing season, but longer-term studies may be needed to clarify the long-term effects on soil and plant.

Core Ideas
- Coal-bed methane production results in coproduced waste water, also called CBMW.
- Sustainable disposal of CBMW is a challenge.
- We determined the effect CBMW on soil and on the biomass and essential oil composition of dill.
- CBMW increased dill ether but reduced carvone in the oil; biomass and oil yields were not affected.
- CBMW increased electrical conductivity and Na but not pH or cation exchange capacity.

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(Mentha spicata L.) and peppermint (Mentha × piperita L.) growth and secondary metabolites. The authors found that these treatments did not change peppermint total phenols, total flavonoids, or antioxidant activity, but herbage yield was affected at higher CBMW rates. Similarly, in spearmint, different levels of CBMW treatment had no significant impact on oil content, oil yield, or antioxidant activity, but there were significant differences in herbage yields. In a 2-yr field study, Burkhardt et al. (2015) compared irrigation with CBMW and municipal (tap) water on yields and ethanol production from corn (Zea mays L.), switchgrass (Panicum virgatum L.), spearmint (Mentha spicata L.), Japanese cornmint (Mentha canadensis L.), lemon-grass (Cymbopogon flexuosus (Nees ex Steud.) J.F. Watson), and common wormwood (Artemisia vulgaris L.). The authors found that the accumulation of plant chemicals (essential oil) in lemon-grass and in spearmint was affected by the use of CBMW. In 2012, CBMW increased the concentration of limonene in Japanese cornmint essential oil and the concentration of α-pinene in common wormwood essential oil, whereas in 2013, CBMW decreased the concentration of citral in lemongrass essential oil. Overall, the authors concluded that CBMW can be used for short-period (2 yr) irrigation of field crops. For longer periods, CBMW may need to be diluted with good-quality water to avoid deleterious effects on soil and crops (Burkhardt et al., 2015).

Dill biomass and essential oil are used in food, beverage, and pharmaceutical products (Larijani et al., 2012; Tian et al., 2012). Previous research suggests that as soil salinity increased to 12 dS m⁻¹, the yield of dill essential oil also increased, possibly because of secretion of secondary metabolites at higher rates during stress (Ghassemi-Golezani et al., 2011). The effect of CBMW alone and with different combinations of tap water on oil content, oil constituents, and fresh biomass of dill has not been studied. Therefore, the objective of this study was to determine the effect of various combinations of CBMW and tap water on the biomass, essential oil yields, essential oil composition, and effect on soil properties of dill grown in a greenhouse, where all parameters can be manipulated.

**Materials and Methods**

**Plant Materials and Growing Conditions**

This experiment was conducted in a greenhouse at the University of Wyoming Sheridan Research and Extension Center. The temperature for the entire period of growth (70 d) was maintained at around 18°C during the night and around 26°C during the daytime; these temperatures are known to be the best growing conditions for dill (Wright, 2005). Humidity was maintained around 50 to 60% for the entire growth period. Dill seeds (‘Bouquet’) were from Ferry Morse Seed Company. Seeds were planted in pots (volume, 11.4 L; diameter, 28 cm) on 19 July 2014. Before planting, drain holes in the bottom of the pots were covered with a double layer of filter paper to prevent soil seepage, and pots were filled with normal field soil (10 kg per pot) from the Sheridan Research and Extension Center at Wyarano site. Fresh and untreated CBMW was hauled weekly from the CBMW treatment site of BeneTerra in Sheridan, WY (Table 1), and irrigation treatments begun on 30 July. There were five different water treatments in six replications. The water

<table>
<thead>
<tr>
<th>pH</th>
<th>8.83</th>
<th>Method used</th>
<th>SM 4500 H B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electrical conductivity, μmhos cm⁻¹</td>
<td>2183</td>
<td>Method used</td>
<td>SM 2510B</td>
</tr>
<tr>
<td>Total dissolved solids (180), mg L⁻¹</td>
<td>1300</td>
<td>Method used</td>
<td>SM 2540</td>
</tr>
<tr>
<td>Alkalinity, total (as CaCO₃), mg L⁻¹</td>
<td>1193</td>
<td>Method used</td>
<td>SM 2320B</td>
</tr>
<tr>
<td>Hardness, Ca/Mg (as CaCO₃), mg L⁻¹</td>
<td>16.3</td>
<td>Method used</td>
<td>SM 2340B</td>
</tr>
<tr>
<td>Nitrogen, ammonia (as N), mg L⁻¹</td>
<td>ND‡</td>
<td>Method used</td>
<td>EPA 350.1</td>
</tr>
<tr>
<td>Sodium adsorption ratio</td>
<td>59.8</td>
<td>Method used</td>
<td>calculation</td>
</tr>
</tbody>
</table>

‡ Not detected.
treatments were as follows: 0% CBMW (tap water only), 25% CBMW (25% CBMW plus 75% tap water), 50% CBMW (50% CBMW plus 50% tap water), 75% CBMW (75% CBMW plus 25% tap water) and 100% CBMW. The soil was a sandy loam with 71% sand, 20% silt, and 9% clay). On the top, 1 to 1.5 cm of commercial growth medium (Sunshine Mix, Sun Gro Horticulture Canada Ltd.) was applied to retain moisture for the germination of dill seeds. Tap water was applied to all of the pots until emergence of dill seedlings; once all pots showed sufficient emergence, seedlings were thinned to 10 vigorous plants per container. At the beginning of the experiment, 600 mL of water was applied every other day. After 1 mo, 600 mL of water was applied daily, but the amount was adjusted during high evaporation or cloudy days to maintain the desired moisture in the soil. For fertilization, urea for nitrogen, murate of potash for potassium, and phosphate for phosphorus were mixed (20–20–20 N–P–K) and applied every 2 wk, maintaining 0.3 g of total mixture per pot. Fertilization was stopped 2 wk before harvest (Joseph and Stephen, 2012). Containers in the greenhouse were moved every 10 to 12 d to achieve complete randomization in the experiment. Dill plants were grown for a total of 70 d. Insecticidal soap (a.i., potassium salt of fatty acid; 10 mL L⁻¹) and malathion (a.i., organophosphate; 10 mL L⁻¹) were used to control aphids on plants.

**Harvesting and Extraction**

Harvesting was done when most of the plants reached flowering stage by hand harvesting of all the plants using secateurs at 4 to 5 cm above the soil surface. Fresh biomass yield was recorded at harvest. Due to insufficient biomass for extraction of essential oil, Rep1 and Rep2, Rep3 and Rep4, and Rep5 and Rep6 of each treatment were combined to form three replications in total. All analyses were performed on these three replications. Essential oil was extracted by cutting the entire dill plants from the combined replications into small pieces (3–4 cm), mixing the pieces in a dishpan, and then selecting 400 g of a biomass sample for distillation. To extract the essential oil, the fresh biomass samples were subjected to steam distillation in a 2-L steam distillation unit (Heartmagic) (Gawde et al., 2009) for 45 min. The essential oil was collected in vials and measured on an analytical scale (model S164, Denver Instrument Co.).

**Soil Sample Analysis**

After completion of the study and immediately after harvest, three replications from each treatment were randomly selected for soil sample analysis. The soil samples were taken from 15 cm depth, dried, and sent to American Agriculture Laboratory Inc., McCook, NE, for analysis. All of the analyses were performed following the procedures as described in Brown (1998). Specifically, soil pH was measured in soil slurry potentiometrically using an electron pH meter (Watson and Brown, 1998), and soil electrical conductivity was measured using 1:1 soil/water dilution method (Whitney, 1998). Soil organic matter was determined using the loss of weight on ignition method (Combs and Nathan, 1998). Potassium was determined by the NCR-13–exchangeable K procedure (Warncke and Brown, 1998), Ca and Mg were determined by atomic absorption, Na was determined by emission (Warncke and Brown, 1998), and cation exchange capacity was calculated using those values.

**Gas Chromatography Flame Ionization Detection: Essential Oil Quantitative Analysis**

Nine constituents were identified and quantified in dill herb essential oil. Essential oil samples were analyzed by gas chromatography flame ionization detection on an Agilent 5975C inert XL MSD with triple axis detector and an Agilent 7693 autosampler (Agilent Technologies, 2010). A DB-5 fused silica capillary column (30 m × 0.25 mm; film thickness, 0.25 μm) was used and operated using the following conditions: injector temperature, 240°C; column temperature, 60 to 120°C at 3°C min⁻¹, then held at 240°C at 20°C min⁻¹ for 5 min (helium was used as carrier); injection volume, 1 μL (split ratio 50:1); flame ionization detector (FID) temperature, 300°C. Postcolumn splitting was performed so that 50% of sample would proceed to FID and 50% to mass spectrometry detection.

The compounds D-limonene, α-pinene, myrcene, α-phellandrene, p-cymene, dill ether, cis-dihydrocarvone, trans-dihydrocarvone, and carvone were identified in the essential oil samples by Kovat analysis (Alencar et al., 1990) and comparison of mass spectra with those reported in the NIST mass spectra database from NIST mass spectral library. Compounds were quantified by performing area percentage calculations based on the total combined FID area. For example, the area for each reported peak was divided by the total integrated area from the FID chromatogram from all reported peaks and multiplied by 100 to arrive at a percentage. The percentage of a peak is a percentage relative to all other constituents integrated in the FID chromatogram.

**Statistical Analysis**

The effects of different levels of CBMW (0, 25, 50, 75, or 100%) on plant height, plant weight, essential oil yield (weight and percent of total oil), essential oil constituents, and various soil properties were analyzed using the GLM procedure of SAS 9.4 (SAS Institute, 2013) for three combined replicates. A completely randomized design was followed. Equality of variances was checked using Hartley’s Table at α 0.05 level of significance, and residuals were verified for normality. Multiple means comparison was performed using Duncan’s multiple range tests at a 5% level of significance. Some values that did not meet equality of variances were transformed to squared root to achieve equality of variances and normality of residuals. To report the results after the analyses, these transformed values were back transformed to the original values by squaring the means of the transformed values.

**Results**

The studied levels of CBMW had varying effects on soil properties and oil constituents. Some of the essential oil constituents (α-pinene, myrcene, α-phellandrene, p-cymene, and cis-dihydrocarvone) and one of the major oil constituents (limonene) were unaffected by the treatment and showed no significant rise or decline in their content. Two other major essential oil constituents, dill ether and carvone, including trans-dihydrocarvone, were influenced by the treatments. Dill ether content was statistically similar at 0 and 25% CBMW and increased at 50% CBMW; there was a slight decrease at 75% CBMW and another increase at 100% CBMW. In contrast, carvone and
trans-dihydrocarvone concentrations were initially high and gradually decreased with increasing levels of CBMW; their levels were lowest at 100% CBMW (Table 2).

Among the analyzed soil variables, only soil pH, cation exchange capacity (which is determined mainly by the soil texture and soil minerals), and base saturation did not change with treatment; all other parameters either increased or decreased with the treatment. Concentration of soluble salts gradually increased with increasing levels of CBMW. In contrast, organic matter content, K, Ca, and Mg concentrations slowly declined with increasing CBMW levels. Base saturation percentages of K, Ca, and Mg followed a similar pattern. Sodium concentration, which is high in CBMW, seemed to increase drastically in soils from the treatments with increased levels of CBMW (Table 3).

Plant height, plant weight, essential oil yield, and essential oil content did not seem to be affected by the treatments. Although essential oil yield and content seemed to decrease with increasing levels of CBMW, they were statistically similar (Table 4).

**Discussion**

Although the CBMW had a high pH (8.83), pH of the soil (6.93–7.26) remained within the best range for plant growth throughout the growing period (Horneck et al., 2011) possibly because of factors such as the short application period of the CBMW treatments (5–6 wk), the use of ammonium and phosphate fertilizers (Frank and Knudsen, 1992; Schroder et al., 2011), and the buffering capacity of soil. Cation exchange capacity and base saturation percentage also did not show significant change with different levels of CBMW and were in the medium range for plants growth (Hill Laboratories, 2010). Soluble salts increased with increasing levels of CBMW and were highest (4.55 mmhos cm$^{-1}$) at 100% CBMW due to the presence of different soluble salts and salt-forming ions (Table 1). Organic matter, K, Ca, and Mg showed a slight decrease with increasing levels of CBMW and were lowest at 100% CBMW. There typically was no organic matter and only trace amounts of Ca and Mg present in the CBMW used in this study (Table 1). The major component of CBMW is Na, and that is why the Na concentration in the soil increased considerably with increasing

### Table 2. Percentage of constituents in dill essential oil at various levels of coal-bed methane water treatment.

<table>
<thead>
<tr>
<th>CBMW†</th>
<th>α-pinene</th>
<th>Myrcene</th>
<th>α-Pheyllandrene</th>
<th>p-Cymene</th>
<th>Limonene</th>
<th>Dill ether</th>
<th>cis-dihydrocarvone</th>
<th>Trans-dihydrocarvone</th>
<th>Carvone</th>
</tr>
</thead>
<tbody>
<tr>
<td>%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.99</td>
<td>0.49</td>
<td>39.9</td>
<td>2.6</td>
<td>22.4</td>
<td>15.2b†</td>
<td>0.19</td>
<td>0.79a</td>
<td>17.4a</td>
</tr>
<tr>
<td>25</td>
<td>1.24</td>
<td>0.53</td>
<td>40.4</td>
<td>4.1</td>
<td>23.1</td>
<td>17.3b</td>
<td>0.14</td>
<td>0.58ab</td>
<td>12.4ab</td>
</tr>
<tr>
<td>50</td>
<td>1.17</td>
<td>0.54</td>
<td>41.3</td>
<td>4.6</td>
<td>22.4</td>
<td>20.3a</td>
<td>0.15</td>
<td>0.5ab</td>
<td>9.0ab</td>
</tr>
<tr>
<td>75</td>
<td>1.14</td>
<td>0.52</td>
<td>39.4</td>
<td>4.1</td>
<td>22.4</td>
<td>17.7b</td>
<td>0.20</td>
<td>0.64ab</td>
<td>12.9ab</td>
</tr>
<tr>
<td>100</td>
<td>1.33</td>
<td>0.57</td>
<td>45.0</td>
<td>5.0</td>
<td>21.8</td>
<td>20.4a</td>
<td>0.05</td>
<td>0.29b</td>
<td>5.2b</td>
</tr>
</tbody>
</table>

† Coal-bed methane water.
‡ Within each column, different lowercase letters denote statistically different values at the 0.05 level of significance (Duncan’s multiple range test).

### Table 3. Soil properties of dill grown at different levels of coal-bed methane water treatments.

<table>
<thead>
<tr>
<th>CBMW†</th>
<th>pH</th>
<th>EC‡</th>
<th>OM§</th>
<th>CEC¶</th>
<th>K</th>
<th>Ca</th>
<th>Mg</th>
<th>Na</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7.20</td>
<td>1.31c#</td>
<td>2.3a</td>
<td>14.66</td>
<td>250a</td>
<td>2257ab</td>
<td>311a</td>
<td>32e</td>
</tr>
<tr>
<td>Initial soil</td>
<td>6.93</td>
<td>2.35bc</td>
<td>2.16ab</td>
<td>15.70</td>
<td>172b</td>
<td>2357a</td>
<td>314a</td>
<td>41e</td>
</tr>
<tr>
<td>0%</td>
<td>6.90</td>
<td>3.52ab</td>
<td>2.06ab</td>
<td>16.23</td>
<td>179b</td>
<td>2283a</td>
<td>299ab</td>
<td>194d</td>
</tr>
<tr>
<td>25%</td>
<td>7.10</td>
<td>3.49ab</td>
<td>2.03b</td>
<td>16.63</td>
<td>168b</td>
<td>2360a</td>
<td>298ab</td>
<td>367c</td>
</tr>
<tr>
<td>50%</td>
<td>7.26</td>
<td>3.33ab</td>
<td>2.16ab</td>
<td>16.00</td>
<td>166b</td>
<td>2147ab</td>
<td>278bc</td>
<td>501b</td>
</tr>
<tr>
<td>75%</td>
<td>7.16</td>
<td>4.55a</td>
<td>2.06ab</td>
<td>15.53</td>
<td>174b</td>
<td>2020b</td>
<td>264c</td>
<td>646a</td>
</tr>
<tr>
<td>100%</td>
<td>7.16</td>
<td>4.55a</td>
<td>2.06ab</td>
<td>15.53</td>
<td>174b</td>
<td>2020b</td>
<td>264c</td>
<td>646a</td>
</tr>
</tbody>
</table>

† Coal-bed methane water.
‡ Electrical conductivity.
§ Organic matter.
¶ Cation exchange capacity.
# Within each column, different lowercase letters denote statistically different values at the 0.05 level of significance (Duncan’s multiple range test).

### Table 4. Plant biomass and oil yield of dill on five coalbed methane water treatments.

<table>
<thead>
<tr>
<th>CBMW†</th>
<th>Plant height</th>
<th>Plant weight</th>
<th>Oil yield</th>
<th>Oil content</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cm</td>
<td>g per combined replication</td>
<td>g per 400 g of fresh biomass</td>
<td>% of fresh wt. (g of oil per 100 g of fresh biomass)</td>
</tr>
<tr>
<td>0%</td>
<td>77.0ab‡</td>
<td>441.6</td>
<td>0.833</td>
<td>0.208</td>
</tr>
<tr>
<td>25%</td>
<td>84.7a</td>
<td>430.0</td>
<td>0.799</td>
<td>0.199</td>
</tr>
<tr>
<td>50%</td>
<td>85.3a</td>
<td>460.0</td>
<td>0.641</td>
<td>0.160</td>
</tr>
<tr>
<td>75%</td>
<td>77.0a</td>
<td>438.3</td>
<td>0.767</td>
<td>0.191</td>
</tr>
<tr>
<td>100%</td>
<td>85.9a</td>
<td>450.0</td>
<td>0.647</td>
<td>0.161</td>
</tr>
</tbody>
</table>

† Coal-bed methane water.
‡ Within each column, different lowercase letters denote statistically different values at the 0.05 level of significance (Duncan’s multiple range test).
levels of CBMW in the treatments. Similar results were observed by Zheljazkov et al. (2013) in a study on spearmint and peppermint; however, their study was conducted using horticultural-grade growth medium (75% Canadian sphagnum peat moss, the rest being horticultural-grade perlite and dolomitic limestone), whereas this study used real field soil.

In this study, the essential oil yield, reported as grams of essential oil per 400 g of fresh biomass, did not change significantly with the application of the different levels of CBMW. In this study, the essential oil content in dill ranged from 0.16 to 0.2%; these values are usually observed in dill, as evident from a study by Porter et al. (1983) where the values for whole-plant oil content of dill ranged from 0.15 to 0.37%. Plant height (77.0–85.9 cm in this study) was similar to the range of 63 to 86 cm found by Zhejljazkov and Warman (2004), and fresh herbage yield (21.5–22.5 g per plant) did not change with the different levels of CBMW. This may be because the level of salts and Na in the soil did not suppress the performance of dill, which is regarded as a crop highly tolerant to soil salinity, and in some cases the dill oil yield seems to increase as salinity stress increases (Ghassemi-Golezani et al., 2011). Similarly, other soil conditions (e.g., pH, cation exchange capacity, and organic matter) were in the optimum range for the plants. Other possible explanations include a relatively short growing cycle (70 d) that minimized CBMW application and good control of other limiting factors, such as disease and pest infestation, irrigation timing, temperature, humidity, and other environmental conditions on which the dill plant depends (Bailer et al., 2001; Sangwan and Farooqi, 2001; Taipoor et al., 2013).

The concentrations of the various essential oil constituents, such as α-pinene (0.99–1.3%), myrcene (0.48–0.57%), α-phellandrene (39.4–45%), limonene (21.8–23.1%), and p-cymene (2.6–5.03%) also did not change among treatments. Similar results (no change) were found for total phenols, total flavonoids, and the antioxidant capacity of peppermint receiving different levels of CBMW (Zheljazkov et al., 2013). The concentrations of the oil constituents in the total essential oil in this study were within the range (wherever available) of those in the study conducted by Callan et al. (2007). Dill ether, one of the major oil constituents of dill essential oil that imparts its characteristic flavor (Blank et al., 1992), seemed to increase with increasing levels of CBMW, with highest ether levels achieved at 50 and 100% CBMW. Other studies have shown increases in secondary metabolite secretions by plants in stressful environments as a defense strategy against various factors. Dill ether, being a major and distinctive oil constituent of dill, is possibly synthesized and accumulated more in stressed environments (Ezz El-Din et al., 2009; Ghassemi-Golezani et al., 2011). Carvone content decreased from 17.4% at 0% CBMW to 5.2% at 100% CBMW, and trans-dihydrocarvone decreased from 0.79% at 0% CBMW to 0.29% at 100% CBMW. A similar response by carvone was observed in peppermint (Zheljazkov et al., 2013). In other studies, anethole and carvone, both of which are antimicrobial compounds (Esfandiyari-Manesh et al., 2013), were observed to decrease with increasing stress levels in soil and the environment. The concentration of carvacrol in oregano and thyme followed a similar decreasing trend with increasing the levels of stress and salinity in soil (Ahl et al., 2009; Ezz El-Din et al., 2009).

The CBMW used in this study contained some Ra (0.3 ± 0.2 pCi L⁻¹), but the concentration of Ra was found to be considerably lower than the USEPA standard for drinking water (≤5 pCi L⁻¹) (Patil et al., 2013) and hence is not a problem in our study. The CBMW also possessed high amounts of Na and some Ba (Table 1), but heavy metals usually remain in plant tissues and do not translocate in essential oils recovered through steam distillation (Scora and Chang, 1997; Zheljazkov and Nielsen, 1996; Zhejljkazov et al., 2006). Other reports demonstrated that essential oil extracted via steam distillation from dill plants contained Cu free even when the soil contained high concentrations of Cu (Zheljazkov and Nielsen, 1996; Scora and Chang, 1997). There are also reports demonstrating that essential oil extracted via steam distillation from dill plants remained unaffected even if the soil had high concentration of some elements, such as Cu (Zheljazkov and Warman, 2004). In this study we found the same pattern (i.e., that high amounts of Na and some Ra and Ba did not seem to affect the oil content). Considering the elevated salts and Na concentrations in the soil treated by different levels of CBMW, soil and plant tissues (residue from steam distillation) may contain high amounts of Na (Zheljazkov and Warman, 2004) and should be disposed of accordingly.

Conclusions

Coal-bed methane water treatments resulted in an increase of dill ether content in the essential oil of dill with increasing the level of CBMW; in contrast, trans-dihydrocarvone and carvone content of the essential oil decreased. Fresh herbage yield and essential oil yield of this plant remained unchanged with different CBMW treatments, so adjustments can be made in the irrigation of this crop to achieve different concentrations of the desired components. However, irrigation with CBMW changes the natural properties of soil. This research only covers the effect of irrigating dill with CBMW on soil that has not previously been exposed to CBMW; further research is needed to ascertain the impact of CBMW irrigation on dill grown in soil previously exposed to CBMW.

Acknowledgments

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