

Harvest Initiated Volatile Organic Compound Emissions from In-Field Tall Wheatgrass

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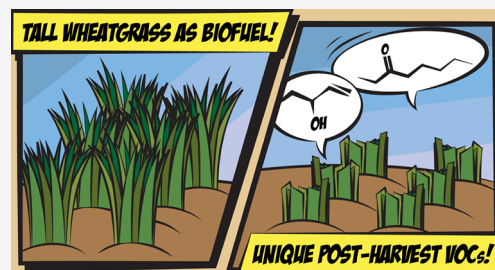
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ABSTRACT: While crop and grassland usage continues to increase, the full diversity of plant-specific volatile organic compounds (VOCs) emitted from these ecosystems, including their implications for atmospheric chemistry and carbon cycling, remains poorly understood. It is particularly important to investigate VOCs in the context of potential biofuels: aside from the implications of large-scale land use, harvest may shift both the flux and speciation of emitted VOCs. To this point, we evaluate the diversity of VOCs emitted both pre and postharvest from “Alkar” tall wheatgrass (*Thinopyrum ponticum*), a candidate biofuel that exhibits greater tolerance to frost and saline land compared to other grass varieties. Mature plants grown under field conditions ($n = 6$) were sampled for VOCs both pre- and postharvest (October 2022). Via hierarchical clustering of emitted VOCs from each plant, we observe distinct “volatilomes” (diversity of VOCs) specific to the pre- and postharvest conditions despite plant-to-plant variability. In total, 50 VOCs were found to be unique to the postharvest tall wheatgrass volatilome, and these unique VOCs constituted a significant portion (26%) of total postharvest signal. While green leaf volatiles (GLVs) dominate the speciation of postharvest emissions (e.g., 54% of unique postharvest VOC signal was due to 1-penten-3-ol), we demonstrate novel postharvest VOCs from tall wheatgrass that are under characterized in the context of carbon cycling and atmospheric chemistry (e.g., 3-octanone). Continuing evaluations will quantitatively investigate tall wheatgrass VOC fluxes, better informing the feasibility and environmental impact of tall wheatgrass as a biofuel.

KEYWORDS: volatile organic compounds, tall wheatgrass, biofuel, carbon cycling, green leaf volatiles



1. INTRODUCTION

Plants produce a vast array of volatile organic compounds (VOCs),^{1–4} which serve as a general indicator of an individual plant’s physiological state. Emitted VOCs are multifunctional, including prominent roles in signaling,^{5–7} stress response,^{8–11} plant lifecycle,^{12,13} and interkingdom interactions.^{14,15} The overall functionality of VOCs is fascinatingly diverse, including examples such as VOCs from insect-attacked maize being emitted to prime nearby plants for future attack,¹⁶ and tobacco plants emitting VOCs resulting from reactions with pest-specific secretions.¹⁷ Significant amounts of carbon may also be introduced into the atmosphere through VOC emissions,^{18,19} resulting in varied atmospheric ozone and secondary organic aerosol formation depending upon the abundance and structure of the emitted VOC.^{20,21} These findings underscore the importance of studying VOCs from specific plant types and community interactions.

Particular plant groups that warrant increased VOC investigation are grasses and crops due to their extensive land coverage: 1244 Mha of land are dedicated to cropland as of 2019,²² and this metric has consistently increased since the 1700s.^{22,23} The importance of understanding these VOCs is compounded by harvest interventions in grass and crop

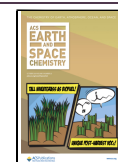
lifecycles, such as in biofuel feedstocks, resulting in highly dynamic VOC abundances and speciation.²⁴ One grass/crop species of interest to the United States Department of Agriculture (USDA) is “Alkar,” a tall wheatgrass (*Thinopyrum ponticum*) (TWG) cultivar that has been evaluated as a potential biofuel feedstock.^{25–27} While a variety of different grasses have been considered as biofuel feedstocks in North America,^{28–30} TWG has been shown to produce the greatest carbohydrate yield among 15 candidate grass species.³¹ TWG is a C3 forage grass species,³² exhibiting a greater tolerance to frost and land types compared to other grass varieties, specifically others that are considered for biofuel feedstocks (e.g., switchgrass, a C4 grass).³³ TWG may be additionally advantageous due to pest resistance, tolerance of/ability to reclaim saline soil, and dual-purpose feasibility as a livestock feed.^{26,27} Despite these inherent advantages of TWG, it is

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imperative to note that large-scale changes in biodiversity (e.g., land reclamation and/or biofuel projects) directly impact climate and overall carbon cycling as a result of emitted VOCs (both pre- and postharvest).^{20,34} As previously mentioned, VOCs participate in the formation of tropospheric ozone and the formation of secondary organic aerosol (SOA); both have adverse health and ecological effects, influencing air quality over local to regional scales. Furthermore, the effects may vary in severity depending on the VOC speciation and abundance,^{11,35} potentially dampening the benefits of TWG as a biofuel feedstock.

The effect of harvest on various grass and crop VOCs has been previously investigated. Damaged cell membranes from physical wounds release polyunsaturated fatty acids. Through the lipoxygenase pathway, VOCs are created from these fatty acids, which are typically five and six carbon-containing alcohols and aldehydes (e.g., (*E*)-2-hexenal), and are often termed “green leaf volatiles” (GLVs).^{19,36–40} Several groups have investigated the atmospheric implications of GLV emissions. As specific examples, secondary organic aerosol formation from *cis*-3-hexenylacetate,^{41,42} *cis*-3-hexen-1-ol,^{41,43} and 1-octen-3-ol⁴⁴ VOC precursors have been previously investigated. But despite what is known, the species specific nature of many VOCs suggests that a holistic characterization of TWG VOC emissions is worthwhile, particularly in response to harvest (i.e., a form of physical plant wounding that may result in significant emissions of GLVs). This may aid in characterizing grass/crop emissions, improve grass/crop VOC modeling approaches, and inform the atmospheric implications of VOC emission from prospective biofuel grasses/crops.

2. MATERIALS AND METHODS

2.1. Plant Growth and Harvest. “Alkar” tall wheatgrass (*Thinopyrum ponticum*) was grown at the Irrigated Tall Wheatgrass Field Trial located at the Washington State University Irrigated Agriculture Research and Extension Center (IAREC) (N 46.25, W 119.73). The trial consists of a test plot of 0.13 ha with 60 subplots averaging 7.9 m² in size. The field site irrigation system is designed to apply 25, 50, 75, and 100% of the field capacity of the soil. TWG in all plots was planted at the same time, such that all plants are of the same age. Three of the 100% irrigation plots were randomly selected. Two plants within each chosen plot (6 plants total) were randomly selected and visually inspected for plant health (i.e., no obvious signs of pest-influence or damage; October 2022). Despite this selection process, it should be acknowledged that minor plant inconsistencies (e.g., minor leaf wounds or pest-influence) may contribute to final plant-to-plant VOC variability given the in-field growth and sampling. Taking care not to damage the plant itself, the plant was covered in a Teflon bag with a sampling port, as shown in Figure 1. Before covering the plant, the Teflon bag was allowed to acclimate to the ambient temperature for ~30 min. An inert stainless steel thermal desorption tube with biomonitoring adsorbent (Markes International Biomonitoring inert coated tubes –1/4 × 3.5”) was connected to the sampling port, and air was pulled through the tube via a pump for 15 min at 375 mL/min (5.6 L total sampled volume, which is below reported breakthrough volumes for common BVOCs with Tenax TA).⁴⁵ Prior to sample collection, thermal desorption tubes were preconditioned for 2.5 h at 335 °C and had brass compression caps for storage and transport to ensure cleanliness. The same sampled plants were subsequently

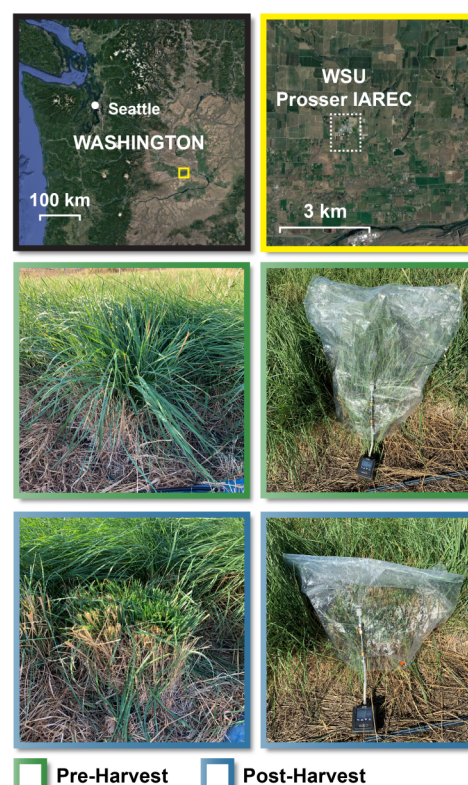


Figure 1. Location of the sampled TWG plants as well as visualization of pre- and postharvest sampling conditions for October 2022.

harvested with a scythe, leaving an 8 in stubble. Within 1 min of harvesting, the same plants were sampled again for VOCs (i.e., postharvest). For both pre- and postharvest samplings, the volume of air within the Teflon bag (i.e., volume of plant subtracted from total volume) was ~2 L for a residence time of ~5 min. All other postharvest sampling parameters were kept identical to the preharvest collection. After sample collection, all sampling tubes were immediately capped and placed in a cooler. All samples were stored at –4 °C prior to analysis. Blank samples were collected both in-field (soil ~15 cm below the surface was sampled without using the Teflon bag to account for interferent soil-derived VOCs) and in-lab (empty Teflon bag to account for interferent sampling system-derived VOCs), with all sampling parameters otherwise identical.

2.2. Gas Chromatography Mass Spectrometry. Samples were analyzed using a Leco Pegasus 4D gas chromatograph by gas chromatography high resolution time-of-flight mass spectrometry system (GC x GC-HRT MS) (LECO Corp., St. Joseph, MI, USA) that was interfaced with a Markes Ultrax thermal desorption unit (TDU) (Markes International, Sacramento CA, USA). The primary GC column used was a Restek Rxi-5 ms (30 m × 0.25 mm × 0.25 μm), and the secondary column used was a Restek Rxt-1701 (1 m × 0.25 mm × 1.00 μm) (Restek Corporation, Bellefonte, PA, USA). The general experimental conditions are listed in Table S1. LECO ChromaTOF software was utilized for data collection (version 5.10) and manual analysis and interpretation (versions 5.10 and 5.55). The electron ionization (EI) mass spectra obtained from the samples were compared to those from a National Institute of Standards and Technology (NIST) database for initial compound assignment. Assignment

of VOC identity is dependent on the similarity of the EI spectrum to standard and appropriate retention time. For all analyses, an internal standard (1 ppm_v toluene-*d*₈) was loaded into a 1 mL gas sample loop in the TDU and injected onto the sample tube prior to tube desorption. Consequently, the internal standard follows the same path as that of the sample when it is desorbed. Further details are described in the [Supporting Information](#).

2.3. Data Processing. VOCs identified via the NIST database were retained if above a signal-to-noise (S/N) threshold of 250. An elevated S/N threshold was used herein, as it is more consistent with the goals of this study and the limited sample set (i.e., confident identification of dominant VOCs representative of TWG). Shannon diversity indexes (H)⁴⁶ were calculated for individual samples (6 preharvest and 6 postharvest samples) according to [eq 1](#), where p_i represents the proportion of total signal area for an individual VOC annotation, and s represents the total number of VOCs annotated in an individual sample.

$$H = -\sum_{i=1}^s p_i \ln p_i \quad (1)$$

All VOC data sets were then aligned into a single file (6 preharvest and 6 postharvest samples for 12 total data sets), where the signal areas were normalized to the toluene-*d*₈ internal standard signal intensity for each sample (accounting for instrumental variability). VOCs were retained if they appeared in data sets for 3 or more plants. Heatmap clustering of the different pre and postharvest TWG samples in this aligned file was then done via the R code “heatmap.2.”⁴⁷ For the sake of heatmap clustering, the logarithm of normalized VOC signal areas was taken. VOCs were removed from final data sets if they appeared in either the in-field or in-lab blank samples.

To create distinct, pre- and postharvest TWG VOC data sets, the VOCs collected from the six replicates were aligned into respective data sets (i.e., 6 preharvest samples were aligned into one list, and 6 postharvest sample VOCs were aligned into a separate list). For greater confidence in the final pre/postharvest VOC lists, a VOC was retained in these lists only if it appeared in at least 4 of 6 TWG samples. Final signals for these VOCs in these pre/postharvest data sets are the average signal area across all 6 replicates. Experimental data has been deposited in an open access data repository (<https://zenodo.org/records/10724746>).

3. RESULTS AND DISCUSSION

3.1. Clustering of Plant VOCs by Harvest Condition.

Our experiment demonstrates a general difference in pre- and postharvest “volatilomes” (full diversity of VOCs) of field grown TWG plants despite variability among the individual replicates. The complete lists of VOCs from each TWG plant both pre and postharvest are tabulated in [Tables S2 and S3](#) and are also shown graphically according to signal areas in [Figures S1](#). Given the untargeted nature of this study, the provided VOC identities are suggested on the basis of NIST library database matching. Therefore, the contents of [Tables S2, S3](#), and [Figure S1](#) display an initial assessment of the TWG volatilome present both before and immediately after harvest. Shannon diversity indexes are also slightly increased for postharvest TWG compared to preharvest for each individual

plant ([Table 1](#)), potentially indicating a general difference in the TWG volatilome dependent upon harvest condition.

Table 1. Shannon Diversity Indexes for Pre- and Postharvest TWG Volatilomes

plant	Shannon diversity index	
	preharvest	postharvest
1	3.36	3.43
2	3.28	3.52
3	3.42	3.52
4	3.40	3.48
5	3.30	3.56
6	3.45	3.49

Hierarchical clustering of the 12 individual data sets (i.e., 6 preharvest and 6 postharvest) was performed next as shown in [Figure 2](#) to discern whether the TWG volatilomes from individual plants would cluster according to distinct harvest conditions. The individual plant samples are represented along the y axis, while the constituent VOCs in all samples are represented along the x axis (select individual VOCs are highlighted for the sake of clarity). An inset histogram on the top left of [Figure 2](#) shows the total count of VOC identities plotted against the logarithm of measured signal areas. [Figure 2](#) shows that the first division in the hierarchical clustering groups 5 preharvest TWG samples together (labeled as “cluster 1”), while 1 preharvest sample is grouped together with the 6 postharvest TWG samples (labeled as “cluster 2”). Therefore, while the results do not cluster exactly according to the harvest condition, it is important to note that these results were obtained in a field setting (more consistent with the goals of assessing TWG biofuel feasibility) and not in a well-controlled laboratory growth scenario. Therefore, given that a suite of factors may lead to plant-to-plant variability (e.g., potential pest related affects that were visibly undetectable), the results shown in [Figure 2](#) still point to discernably different TWG volatilomes according to harvest condition. This plant-to-plant variability is further visualized in [Figure 3](#), where the total signal areas for all detected VOCs are summed per TWG sample (displayed on the primary axis). The dried harvested plant biomass is also presented via a secondary axis, showing a comparatively higher harvested biomass for plants 1 and 3 compared to 3 through 6. However, each plant pair (i.e., pre- and postharvest samples) still shows a consistent increase in total signal area for the postharvest condition relative to preharvest despite the loss of biomass and the plant-to-plant variability via varied summed signal intensities. It is furthermore notable that the plant 2 preharvest TWG sample, shown via [Figure 3](#) to have the highest signal among all preharvest samples, was the one that clustered with the postharvest samples in [Figure 2](#). Overall, [Figures 2 and 3](#) represent an initial assessment of the TWG volatilome and highlight a discernible shift in the volatilome after harvest for TWG.

3.2. Comparing Emissions for Individual VOCs. The difference in pre- and postharvest volatilomes of TWG may alternatively be visualized in [Figure 4](#). For each individual plant ($n = 6$), the preharvest signal was subtracted from the postharvest signal, such that a positive value is indicative of greater signal postharvest (and by extension, a negative value is indicative of greater signal preharvest). [Figure 4](#) displays the averages and standard deviations of these paired differences for

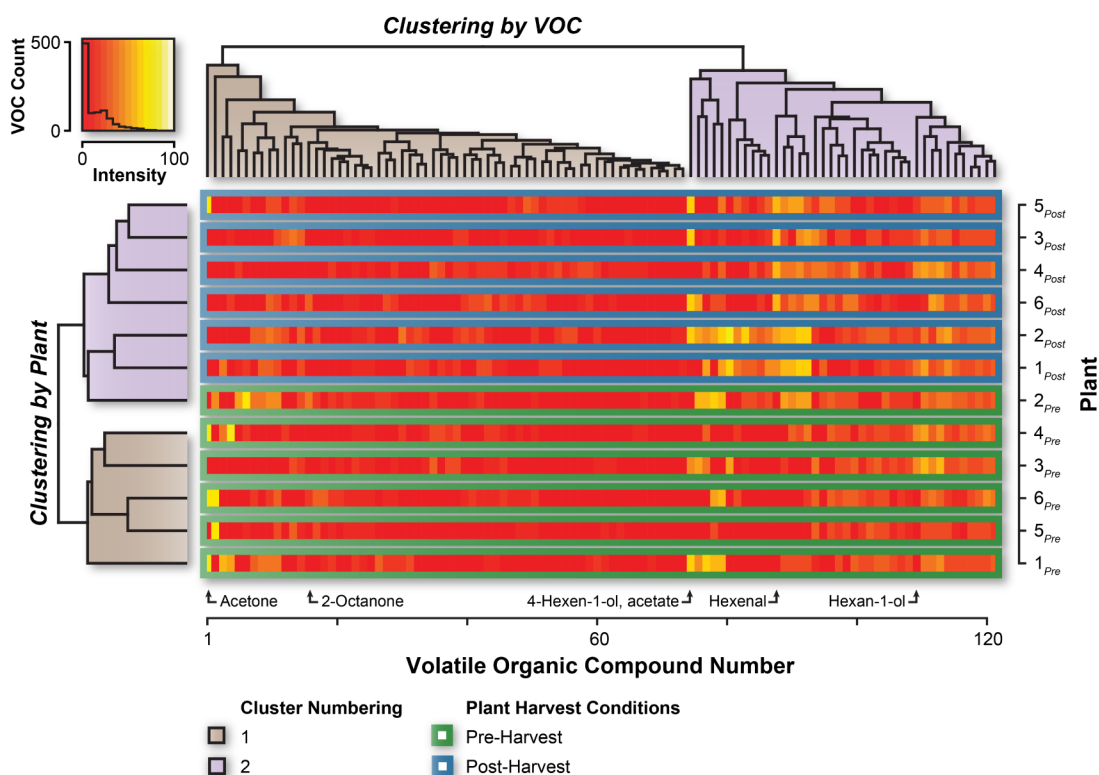


Figure 2. Hierarchical clustering of preharvest ($n_{\text{pre}} = 6$) and postharvest ($n_{\text{post}} = 6$) TWG plants based upon emitted VOCs. Displayed signal intensities (i.e., “value” as indicated in the legend) are indicative of the logarithm of signal area. Count refers to the number of VOCs. Labeled VOCs are suggested annotations from the NIST database.

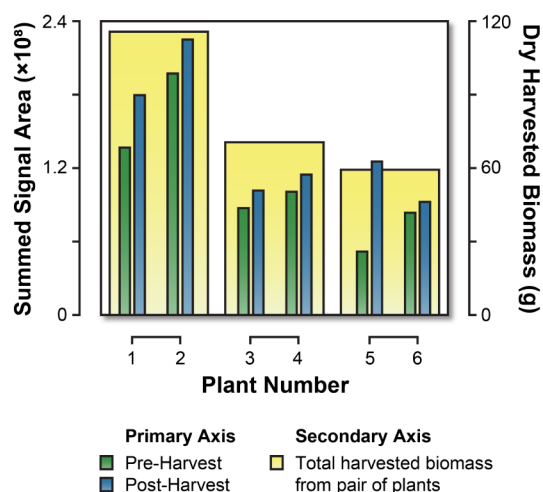


Figure 3. Summed signal areas for VOCs emitted from preharvest ($n_{\text{pre}} = 6$) and postharvest ($n_{\text{post}} = 6$) TWG plants (displayed on primary y-axis). Additionally shown are totals of dry harvested biomass for pairs of plants (displayed on the secondary y-axis).

the top 20 overexpressed postharvest and top 20 overexpressed preharvest VOCs detected from TWG as suggested by the NIST database. While plant-to-plant variability is again apparent, Figure 4 provides a clear visualization of which VOCs drive the different volatiles according to harvest condition. It is apparent via Figure 4 that the postharvest TWG volatilome is dominated by GLVs (individual VOCs labeled on Figure 4).^{19,37} For example, the top 6 postharvest signal increase VOCs are all GLVs or derivatives (e.g., hexanal), while several prominent preharvest VOCs are also GLVs (e.g., 3-

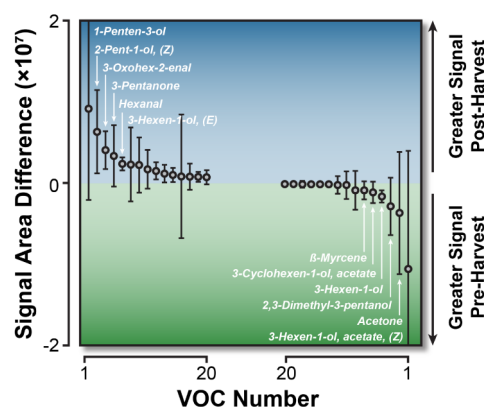


Figure 4. Pair-wise averages of signal area differences (preharvest signal area subtracted from postharvest signal area for individual plant; $n = 6$) plotted against VOC number for TWG samples. The top 20 overexpressed postharvest and top 20 overexpressed preharvest VOCs are shown, with the top 6 from each harvest conditions labeled for clarity. Error bars represent the uncertainty in the pairwise signal area differences. Labeled VOCs are suggested annotations from the NIST database.

hexen-1-ol). This is alternatively visualized in Figure 5, which shows a distinct increase in signal for C_5 and C_6 species due to harvest compared to the preharvest condition, which is synonymous with GLVs. Given the untargeted nature of this investigation, it must again be caveated that there is potential for some of the postulated VOCs to be incorrectly identified, given the reliance on database matching. The accuracy and depth of interpretation of the information communicated through Figures 4 and 5 may also be improved by the broader

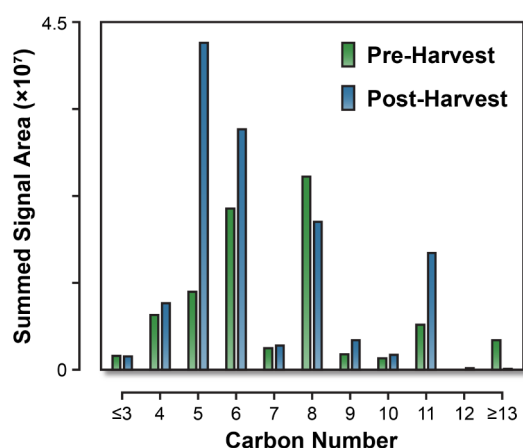


Figure 5. Summed signal areas for VOCs emitted from preharvest ($n_{\text{Pre}} = 6$) and postharvest ($n_{\text{Post}} = 6$) TWG plants according to carbon number.

usage of authentic standard comparisons. However, despite these limitations, the initial untargeted assessments of the TWG shifting volatilome as a result of harvest presented here nevertheless do not deviate from what has been expected from other grass species.^{10,19,37}

While Figures 4 and 5 are useful first means of visualizing the changing volatilome of TWG due to harvest, the dominance of GLVs with respect to changing emissions may mask the evolution of more minor types of VOCs. Therefore, to interpret pre vs postharvest volatilome differences with greater confidence, stricter rules were applied for VOC exclusion: separate pre and postharvest data sets were created, but a VOC was only retained if it appeared in at least 4 of the 6 individual plants data sets. These 2 data sets were then compared against each other for unique features, allowing for an assessment of unique VOCs for each harvest condition. The results of this data analysis are presented in Figure 6, which

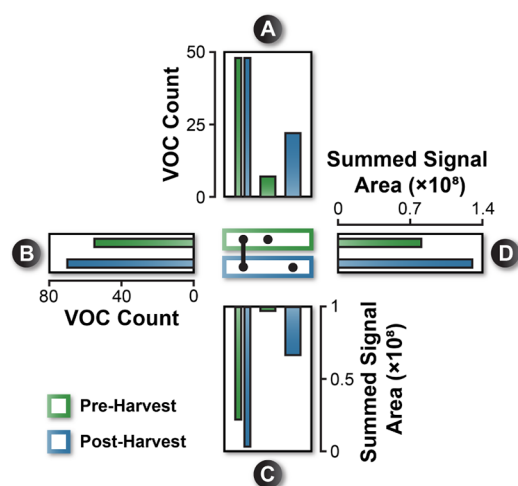


Figure 6. Modified UpSet plot⁴⁸ for comparing both identity and signal area intensity of VOCs emitted from preharvest ($n_{\text{Pre}} = 6$) and postharvest ($n_{\text{Post}} = 6$) TWG plants. For pre- and postharvest TWG data sets. (A) Comparison of VOCs by compound count according to unique or common detection. (B) Comparison of VOCs by compound count. (C) Comparison of VOCs by summed signal area according to unique or common detection. (D) Comparison of VOCs by summed signal area.

contains a modified UpSet plot (similar to Venn diagram).⁴⁸ The legend of this plot is contained in the center, guiding the interpretation of information that is both shared between pre- and postharvest samples as well as unique to each harvest condition (vertical plots; Figures A and C). Figure 6 also preserves the presentation of data set totals, not explicitly accounting for information that is common between harvest conditions (horizontal plots; Figures B and D). Figure 6A shows that 50 VOCs are shared between pre- and postharvest TWG plants, while 7 are unique to preharvest and 22 are unique to postharvest. The total VOC numbers for these constructed pre (55) and postharvest (70) data sets are shown in Figure 6B. Figure 6C shows that 26% of the total postharvest signal intensity results from VOCs that are truly unique to the postharvest condition, whereas the unique preharvest VOCs contribute largely insignificant signal (3% of total preharvest signal). Figure 6D shows a summation of the information previously presented in Figure 3, showing higher overall emissions postharvest compared to preharvest. The increased postharvest signal is particularly significant given the decrease in biomass as compared to the preharvest condition (Figures 1 and 3). Therefore, due to the confident observation that harvest shifts the TWG volatilome to a suite of VOCs, the unique postharvest VOCs will be discussed in the context of atmospheric and biofuel usage implications.

3.3. Biofuel Feedstock Production and Atmospheric Implications. It has previously been noted that VOCs contribute to ozone and SOA production,³⁵ and it has also been noted before that VOC functional group may be a pivotal factor in atmospheric lifetime/evolution.^{49–52} To this point, Figure 7 shows the changes in VOC number and summed signal intensity between pre- and postharvest conditions (from the same data sets constructed for Figure 6) according to organic functional group. Figure 7 shows that postharvest emission increases are dominated by carbonyl (30 total VOCs; 12 VOCs unique to postharvest; 18% of postharvest carbonyl emission due to unique VOCs) and alcohol (13 total VOCs; 5 VOCs unique to postharvest; 48% of postharvest alcohol emission due to unique VOCs) species. While the atmospheric evolution of VOCs are dependent on more than functional group alone (e.g., aromaticity is a key factor for photolysis),¹⁸ the dominance of oxygenated VOCs (carbonyls, alcohols) here suggests that aqueous phase removal/reactions (due to increased aqueous solubility) may have significant rates compared to gas-phase processes.^{19,53}

Bar charts for the unique pre- and postharvest VOC proportional signal area contributions are additionally shown in Figure 7. While specific unique preharvest VOCs are indeed noted, Figure 7 again highlights their overall minimal contribution (3% of total preharvest signal area) compared to the preharvest volatilome as a whole. There is furthermore the possibility that low intensity unique preharvest VOCs may be misannotated (e.g., 1,3-dimethyl-benzene is suggested as a minor unique preharvest VOC but may not be properly identified via the NIST database) and should therefore be cautiously interpreted. Despite this obvious limitation of untargeted analysis, some notable unique preharvest alkanes (e.g., hexadecane, emitted by the plant itself or by symbiotic microbes) have been observed, and such VOCs have previously been linked to specific pathogen resistance.^{54–56} Therefore, given that these VOCs are unique to the preharvest condition, it is possible that the harvest process resulted in a shift of plant energy prioritization to alternative defense

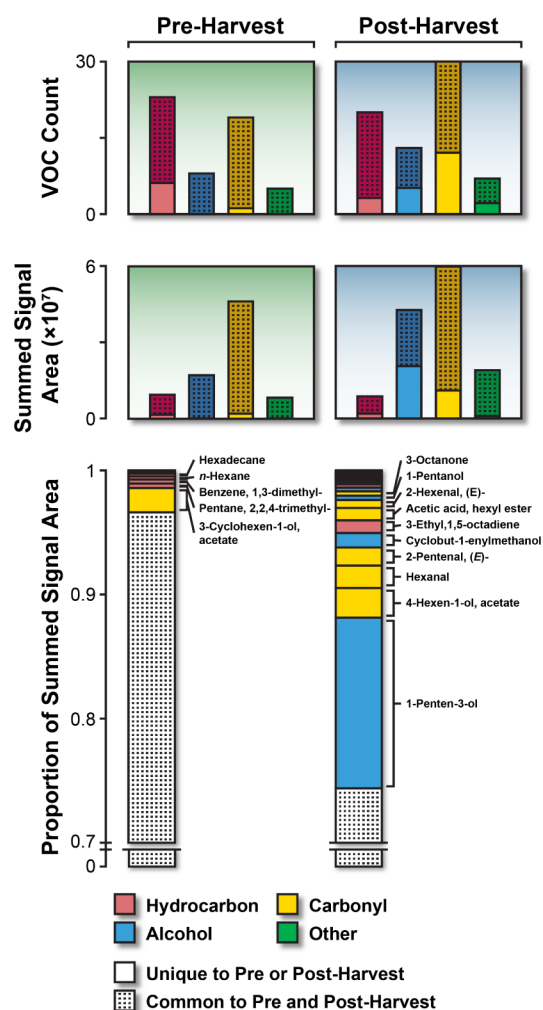


Figure 7. Number of VOCs and summed signal areas for VOCs emitted from preharvest ($n_{\text{pre}} = 6$) and postharvest ($n_{\text{post}} = 6$) TWG plants according to functional group (hydrocarbon, carbonyl, alcohol, other). Additionally shown below are pie charts detailing the proportion of summed signal areas of unique pre- and postharvest VOCs compared to the samples as a whole. Labeled VOCs are suggested annotations from the NIST database.s

strategies. Specifically, in the case of the unique postharvest VOCs, eight of the top ten by signal area proportion are GLVs. Figure 7, in particular, highlights the elevated contributions of the GLV 1-penten-3-ol (54% of unique postharvest signal area, which is in alignment with the significant increase in postharvest C_5 signal as shown in Figure 5). While GLVs have been more broadly investigated for SOA formation potential,^{19,41–44} several studies have specifically focused on 1-penten-3-ol, including those that highlight its role in new particle formation,⁵⁷ loss through photolysis and hydroxyl radicals reactions,^{58,59} and comparative kinetics with other GLVs.⁵³ Although not a major signal, 3-octanone (not a GLV) appears in the postharvest TWG data set (1% of postharvest signal area) and was confirmed via comparison with an authentic standard. Multiple species across kingdoms are known to emit 3-octanone to kill pests/prey,^{60,61} and it may therefore be uniquely emitted postharvest here by TWG as an additional defense mechanism additive to other GLV defense effects.^{19,39} Despite the overall minor abundance according to the signal area, non-GLV VOCs such as 3-octanone may play

profound roles in SOA formation. To this point, Harvey et al. predicted SOA mass yield from freshly cut grass GLV VOCs but found that the experimental SOA mass yields exceeded the predicted amount by ~150%.⁶² Therefore, additional VOCs beyond dominant GLVs, including potentially 3-octanone, may result in a significant SOA yield. Future assessments will involve calibrating the 3-octanone signal postharvest, allowing for a quantitative emission assessment.

While the results herein are qualitative and semiquantitative (on the basis of uncalibrated signal areas), the quantitative results may be roughly estimated by comparisons to work from Eller et al., who have profiled the VOC emissions from mature switchgrass cultivars (C4 grass species).³³ Therein, Eller et al. found high qualitative similarity in the VOCs for switchgrass compared to what is found here for TWG (e.g., dominance of 1-penten-3-ol emission postharvest). In their study, it was estimated that the global SOA burden related to switchgrass growth/harvest for biofuels is 0.03 Tg yr⁻¹, but this represents only ~0.003% of estimated global BVOC emissions (1087 Tg yr⁻¹).^{19,63} Importantly, these emissions are markedly lower (and expected to be lower for other related grasses such as TWG) compared to other nongrass biofuel candidates, such as *Eucalyptus*⁶⁴ and tree varieties.^{65,66} Therefore, combining the TWG advantages associated with climate, land tolerance (classification as C3 grass species),^{26,27} and high carbohydrate yield,³¹ the findings herein regarding the TWG volatilome further promote the prospective biofuel usage of TWG with minimized environmental impact.

4. CONCLUSIONS

This study reveals the volatilome of TWG, addressing knowledge gaps associated with VOCs of grasses in general, as well as the prospective use of TWG as a biofuel feedstock. Continuing studies will quantitatively assess the TWG volatilome, including over multiple harvests and varied external stressors (e.g., drought). These investigations may also be better targeted toward specific VOCs, informed by the initial untargeted work here. The atmospheric implications may also be investigated further via monitoring of VOCs over greater timespans (e.g., time-dependent fluxes and novel VOC emission) as well as examining novel roles of VOCs in SOA and ozone formation. While the results herein are complementary of TWG usage for biofuel, it is important to reemphasize the interconnected nature of terrestrial and atmospheric carbon cycles.²⁰ While increased crop coverage may lead to the removal of atmospheric carbon (i.e., carbon farming)⁶⁷ and alternative energy sources (i.e., biofuel), frequent harvests may reintroduce a significant portion of the same carbon originally fixed by photosynthesis (potentially up to 10%) back to the atmosphere.⁶⁸ It must also be noted that changing land use may alter the Earth's albedo, potentially resulting in a net warming effect.⁶⁹ Therefore, while this study addresses one portion of prospective biofuel usage (specifically, the volatilome), the investigation of biofuel viability should continue.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsearthspacechem.4c00046>.

Complete VOC lists for pre and postharvest TWG plants, VOCs by average signal area, top pre and postharvest VOCs (PDF)

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Notes

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REFERENCES

- (1) Liu, Z.; Wang, M.; Wu, M.; Li, X.; Liu, H.; Niu, N.; Li, S.; Chen, L. Volatile Organic Compounds (VOCs) from Plants: From Release to Detection. *TrAC, Trends Anal. Chem.* **2023**, *158*, 116872.
- (2) Vivaldo, G.; Masi, E.; Taiti, C.; Caldarelli, G.; Mancuso, S. The Network of Plants Volatile Organic Compounds. *Sci. Rep.* **2017**, *7* (1), 11050.
- (3) Tholl, D.; Hossain, O.; Weinhold, A.; Röse, U. S. R.; Wei, Q. Trends and Applications in Plant Volatile Sampling and Analysis. *Plant J.* **2021**, *106* (2), 314–325.
- (4) Knudsen, J. T.; Eriksson, R.; Gershenzon, J.; Ståhl, B. Diversity and Distribution of Floral Scent. *Bot. Rev.* **2006**, *72* (1), 1.
- (5) Baldwin, I. T.; Halitschke, R.; Paschold, A.; von Dahl, C. C.; Preston, C. A. Volatile Signaling in Plant-Plant Interactions: “Talking Trees the Genomics Era. *Science* **2006**, *311* (5762), 812–815.
- (6) Heil, M.; Karban, R. Explaining Evolution of Plant Communication by Airborne Signals. *Trends Ecol. Evol.* **2010**, *25* (3), 137–144.
- (7) Ninkovic, V.; Markovic, D.; Rensing, M. Plant Volatiles as Cues and Signals in Plant Communication. *Plant Cell Environ.* **2021**, *44* (4), 1030–1043.
- (8) Faiola, C. L.; Pullinen, I.; Buchholz, A.; Khalaj, F.; Ylisirniö, A.; Kari, E.; Miettinen, P.; Holopainen, J. K.; Kivimäenpää, M.; Schobesberger, S.; Yli-Juuti, T.; Virtanen, A. Secondary Organic Aerosol Formation from Healthy and Aphid-Stressed Scots Pine Emissions. *ACS Earth Sp. Chem.* **2019**, *3* (9), 1756–1772.
- (9) Fall, R.; Karl, T.; Hansel, A.; Jordan, A.; Lindinger, W. Volatile Organic Compounds Emitted after Leaf Wounding: On-Line Analysis by Proton-Transfer-Reaction Mass Spectrometry. *J. Geophys. Res.: Atmos.* **1999**, *104* (D13), 15963–15974.
- (10) Brilli, F.; Hörtnagl, L.; Bamberger, I.; Schnitzhofer, R.; Ruuskanen, T. M.; Hansel, A.; Loreto, F.; Wohlfahrt, G. Qualitative and Quantitative Characterization of Volatile Organic Compound Emissions from Cut Grass. *Environ. Sci. Technol.* **2012**, *46* (7), 3859–3865.
- (11) Faiola, C. L.; Jobson, B. T.; VanReken, T. M. Impacts of Simulated Herbivory on Volatile Organic Compound Emission Profiles from Coniferous Plants. *Biogeosciences* **2015**, *12* (2), 527–547.
- (12) Gomez, L. G.; Loubet, B.; Lafouge, F.; Ciuraru, R.; Bsaibes, S.; Kammer, J.; Buysse, P.; Durand, B.; Gueudet, J. C.; Fanucci, O.; et al. Effect of Senescence on Biogenic Volatile Organic Compound Fluxes in Wheat Plants. *Atmos. Environ.* **2021**, *266*, 118665.
- (13) Mozaffar, A.; Schoon, N.; Bachy, A.; Digrado, A.; Heinesch, B.; Aubinet, M.; Fauconnier, M.-L.; Delaplace, P.; du Jardin, P.; Amelynck, C. Biogenic Volatile Organic Compound Emissions from Senescent Maize Leaves and a Comparison with Other Leaf Developmental Stages. *Atmos. Environ.* **2018**, *176*, 71–81.
- (14) Poveda, J. Beneficial Effects of Microbial Volatile Organic Compounds (MVOs) in Plants. *Appl. Soil Ecol.* **2021**, *168*, 104118.
- (15) Duc, N. H.; Vo, H. T. N.; van Doan, C.; Hamow, Le, K. H.; Posta, K. Volatile organic compounds shape belowground plant–fungi interactions. *Front. Plant Sci.* **2022**, *13*, 1046685.
- (16) Ton, J.; D’Alessandro, M.; Jourdie, V.; Jakab, G.; Karlen, D.; Held, M.; Mauch-Mani, B.; Turlings, T. C. J. Priming by Airborne Signals Boosts Direct and Indirect Resistance in Maize. *Plant J.* **2007**, *49* (1), 16–26.
- (17) Allmann, S.; Baldwin, I. T. Insects Betray Themselves in Nature to Predators by Rapid Isomerization of Green Leaf Volatiles. *Science* **2010**, *329* (5995), 1075–1078.
- (18) Atkinson, R. Atmospheric Chemistry of VOCs and NOx. *Atmos. Environ.* **2000**, *34* (12), 2063–2101.
- (19) Sarang, K.; Rudziński, K. J.; Szmigielski, R. Green Leaf Volatiles in the Atmosphere—Properties, Transformation, and Significance. *Atmosphere* **2021**, *12*, 1655.
- (20) Holopainen, J. K.; Kivimäenpää, M.; Nizkorodov, S. A. Plant-Derived Secondary Organic Material in the Air and Ecosystems. *Trends Plant Sci.* **2017**, *22* (9), 744–753.
- (21) Shrivastava, M.; Cappa, C. D.; Fan, J.; Goldstein, A. H.; Guenther, A. B.; Jimenez, J. L.; Kuang, C.; Laskin, A.; Martin, S. T.; Ng, N. L.; Petaja, T.; Pierce, J. R.; Rasch, P. J.; Roldin, P.; Seinfeld, J. H.; Shilling, J.; Smith, J. N.; Thornton, J. A.; Volkamer, R.; Wang, J.; Worsnop, D. R.; Zaveri, R. A.; Zelenyuk, A.; Zhang, Q. Recent Advances in Understanding Secondary Organic Aerosol: Implications for Global Climate Forcing. *Rev. Geophys.* **2017**, *55* (2), 509–559.
- (22) Potapov, P.; Turubanova, S.; Hansen, M. C.; Tyukavina, A.; Zalles, V.; Khan, A.; Song, X.-P.; Pickens, A.; Shen, Q.; Cortez, J. Global Maps of Cropland Extent and Change Show Accelerated Cropland Expansion in the Twenty-First Century. *Nat. Food* **2022**, *3* (1), 19–28.

- (23) Ellis, E. C.; Klein Goldewijk, K.; Siebert, S.; Lightman, D.; Ramankutty, N. Anthropogenic Transformation of the Biomes, 1700 to 2000. *Glob. Ecol. Biogeogr.* **2010**, *19* (5), 589–606.
- (24) Kirstine, W.; Galbally, I.; Ye, Y.; Hooper, M. Emissions of Volatile Organic Compounds (Primarily Oxygenated Species) from Pasture. *J. Geophys. Res.: Atmos.* **1998**, *103* (D9), 10605–10619.
- (25) Ciria, C. S.; Sastre, C. M.; Carrasco, J.; Ciria, P. Tall Wheatgrass (*Thinopyrum Ponticum* (Podp)) in a Real Farm Context, a Sustainable Perennial Alternative to Rye (*Secale Cereale* L.) Cultivation in Marginal Lands. *Ind. Crops Prod.* **2020**, *146*, 112184.
- (26) USDA-NRCS Plant Materials Center. *Release Brochure for Alkar Tall Wheatgrass (Thinopyrum Ponticum)*; USDA-NRCS Plant Materials Center, 2022.
- (27) Briggs, J.; Salon, P.; Leif, L.; Durling, R.; St John, L.; Tilley, D.; Young-Mathews, A.; Smith, C.; Plumb, H.; Williams, J., et al. *Evaluation of Tall Wheatgrass Cultivars as a Biofuel Feedstock in Cool Season Grass Regions of the USA*; USDA, 2020.
- (28) Crespo, E.; Graus, M.; Gilman, J. B.; Lerner, B. M.; Fall, R.; Harren, F. J. M.; Warneke, C. Volatile Organic Compound Emissions from Elephant Grass and Bamboo Cultivars Used as Potential Bioethanol Crop. *Atmos. Environ.* **2013**, *65*, 61–68.
- (29) Dickeduisberg, M.; Laser, H.; Tonn, B.; Isselstein, J. Tall Wheatgrass (*Agropyron Elongatum*) for Biogas Production: Crop Management More Important for Biomass and Methane Yield than Grass Provenance. *Ind. Crops Prod.* **2017**, *97*, 653–663.
- (30) Larson, S. R.; Pearson, C. H.; Jensen, K. B.; Jones, T. A.; Mott, I. W.; Robbins, M. D.; Staub, J. E.; Waldron, B. L. Development and Testing of Cool-Season Grass Species, Varieties and Hybrids for Biomass Feedstock Production in Western North America. *Agronomy* **2017**, *7*, 3.
- (31) Payne, C.; Wolfrum, E. J.; Nagle, N.; Brummer, J. E.; Hansen, N. Evaluation of Fifteen Cultivars of Cool-Season Perennial Grasses as Biofuel Feedstocks Using Near-Infrared. *Agron. J.* **2017**, *109* (5), 1923–1934.
- (32) Borrajo, C. I.; Sánchez-Moreiras, A. M.; Reigosa, M. J. Ecophysiological Responses of Tall Wheatgrass Germplasm to Drought and Salinity. *Plants* **2022**, *11*, 1548.
- (33) Eller, A. S. D.; Sekimoto, K.; Gilman, J. B.; Kuster, W. C.; de Gouw, J. A.; Monson, R. K.; Graus, M.; Crespo, E.; Warneke, C.; Fall, R. Volatile Organic Compound Emissions from Switchgrass Cultivars Used as Biofuel Crops. *Atmos. Environ.* **2011**, *45* (19), 3333–3337.
- (34) Sanaei, A.; Herrmann, H.; Alshaabi, L.; Beck, J.; Ferlian, O.; Fomba, K. W.; Haferkorn, S.; van Pinxteren, M.; Quaa, J.; Quosh, J.; et al. Changes in Biodiversity Impact Atmospheric Chemistry and Climate through Plant Volatiles and Particles. *Commun. Earth Environ.* **2023**, *4* (1), 445.
- (35) Gu, S.; Guenther, A.; Faiola, C. Effects of Anthropogenic and Biogenic Volatile Organic Compounds on Los Angeles Air Quality. *Environ. Sci. Technol.* **2021**, *55* (18), 12191–12201.
- (36) Liavonchanka, A.; Feussner, I. Lipoxygenases: Occurrence, Functions and Catalysis. *J. Plant Physiol.* **2006**, *163* (3), 348–357.
- (37) Matsui, K.; Koeduka, T. Green Leaf Volatiles in Plant Signaling and Response, In *Lipids in Plant and Algae Development*, Nakamura, Y.; Li-Beisson, Y., Eds.; Springer International Publishing: Cham, 2016, pp. 427443, .
- (38) Bate, N. J.; Rothstein, S. J. C6-Volatiles Derived from the Lipoxygenase Pathway Induce a Subset of Defense-Related Genes. *Plant J.* **1998**, *16* (5), 561–569.
- (39) Matsui, K.; Engelberth, J. Green Leaf Volatiles—The Forefront of Plant Responses Against Biotic Attack. *Plant Cell Physiol.* **2022**, *63* (10), 1378–1390.
- (40) Mochizuki, S.; Sugimoto, K.; Koeduka, T.; Matsui, K. Arabidopsis Lipoxygenase 2 Is Essential for Formation of Green Leaf Volatiles and Five-Carbon Volatiles. *FEBS Lett.* **2016**, *590* (7), 1017–1027.
- (41) Hamilton, J. F.; Lewis, A. C.; Carey, T. J.; Wenger, J. C.; Borrás i Garcia, E.; Muñoz, A. Reactive Oxidation Products Promote Secondary Organic Aerosol Formation from Green Leaf Volatiles. *Atmos. Chem. Phys.* **2009**, *9* (11), 3815–3823.
- (42) Shi, D.; Liu, J.; Wang, Y.; Xu, L.; Guo, T.; Jia, B.; Cheng, P. Secondary Organic Aerosol Formation from Cis-3-Hexen-1-ol/NO_x Photo-Oxidation: The Roles of Cis-3-Hexen-1-ol Concentration, Illumination Intensity, NO_x and NH₃. *Atmos. Environ.* **2022**, *278*, 119090.
- (43) Shi, D.; Chen, Y.; Liu, J.; Zhao, G.; Xu, L.; Jia, B.; Cheng, P. Formation of Secondary Organic Aerosols from the Ozonolysis of Cis-3-Hexenyl Acetate: The Effect of Acidic Seed Particles and SO₂. *Atmos. Environ.* **2023**, *309*, 119907.
- (44) Fischer, K. B.; Gold, C. S.; Harvey, R. M.; Petrucci, A. N.; Petrucci, G. A. Ozonolysis Chemistry and Phase Behavior of 1-Octen-3-ol-Derived Secondary Organic Aerosol. *ACS Earth Sp. Chem.* **2020**, *4* (8), 1298–1308.
- (45) Hellén, H.; Tykkä, T.; Schallhart, S.; Stratigou, E.; Salameh, T.; Iturrate-Garcia, M. Measurements of Atmospheric C₁₀–C₁₅ Biogenic Volatile Organic Compounds (BVOCs) with Sorbent Tubes. *Atmos. Meas. Technol.* **2024**, *17* (1), 315–333.
- (46) Wetzol, W. C.; Whitehead, S. R. The Many Dimensions of Phytochemical Diversity: Linking Theory to Practice. *Ecol. Lett.* **2020**, *23* (1), 16–32.
- (47) Warnes, G. R.; Bolker, B.; Bonebakker, L.; Gentleman, R.; Huber, W.; Liaw, A.; Lumley, T.; Maechler, M.; Magnusson, A.; Moeller, S., et al. *Gplots: Various R Programming Tools for Plotting Data*. *R Package Version 2.17.0*, ScienceOpen, Inc. 2015.
- (48) Conway, J. R.; Lex, A.; Gehlenborg, N. UpSetR: An R Package for the Visualization of Intersecting Sets and Their Properties. *Bioinformatics* **2017**, *33* (18), 2938–2940.
- (49) Hou, G.-L.; Lin, W.; Wang, X.-B. Direct Observation of Hierarchic Molecular Interactions Critical to Biogenic Aerosol Formation. *Commun. Chem.* **2018**, *1* (1), 37.
- (50) Zhang, X.; Seinfeld, J. H. A Functional Group Oxidation Model (FGOM) for SOA Formation and Aging. *Atmos. Chem. Phys.* **2013**, *13* (12), 5907–5926.
- (51) Jia, L.; Xu, Y. The Role of Functional Groups in the Understanding of Secondary Organic Aerosol Formation Mechanism from α -Pinene. *Sci. Total Environ.* **2020**, *738*, 139831.
- (52) Claflin, M. S.; Krechmer, J. E.; Hu, W.; Jimenez, J. L.; Ziemann, P. J. Functional Group Composition of Secondary Organic Aerosol Formed from Ozonolysis of α -Pinene Under High VOC and Autoxidation Conditions. *ACS Earth Sp. Chem.* **2018**, *2* (11), 1196–1210.
- (53) Sarang, K.; Otto, T.; Rudzinski, K.; Schaefer, T.; Grgić, I.; Nestorowicz, K.; Herrmann, H.; Szmigielski, R. Reaction Kinetics of Green Leaf Volatiles with Sulfate, Hydroxyl, and Nitrate Radicals in Tropospheric Aqueous Phase. *Environ. Sci. Technol.* **2021**, *55* (20), 13666–13676.
- (54) Bee Park, H.; Lee, B.; Kloepper, J. W.; Ryu, C.-M. One Shot-Two Pathogens Blocked. *Plant. Signal. Behav.* **2013**, *8* (7), No. e24619.
- (55) Sheikh, T. M. M.; Zhou, D.; Ali, H.; Hussain, S.; Wang, N.; Chen, S.; Zhao, Y.; Wen, X.; Wang, X.; Zhang, J.; et al. Volatile Organic Compounds Emitted by the Biocontrol Agent *Pythium Oligandrum* Contribute to Ginger Plant Growth and Disease Resistance. *Microbiol. Spectr.* **2023**, *11* (4), No. e01510–23.
- (56) Wang, K.; Ke, S.; Fang, W.; Liu, F.; Zhang, Z. Agroactive Volatile Organic Compounds from Microbes: Chemical Diversities and Potentials of Application in Crop Protection. *Adv. Agrochem.* **2023**, *2* (1), 39–57.
- (57) O Dwyer, M. A.; Carey, T. J.; Healy, R. M.; Wenger, J. C.; Picquet-Varrault, B.; Doussin, J. F. The Gas-Phase Ozonolysis of 1-Penten-3-ol, (Z)-2-Penten-1-ol and 1-Penten-3-one: Kinetics, Products and Secondary Organic Aerosol Formation. *Zeitschrift für Physikalische Chemie* **2010**, *224* (7–8), 1059–1080.
- (58) Jiménez, E.; Lanza, B.; Antiñolo, M.; Albaladejo, J. Photo-oxidation of Leaf-Wound Oxygenated Compounds, 1-Penten-3-ol, (Z)-3-Hexen-1-ol, and 1-Penten-3-one, Initiated by OH Radicals and Sunlight. *Environ. Sci. Technol.* **2009**, *43* (6), 1831–1837.
- (59) Sarang, K.; Otto, T.; Gagan, S.; Rudzinski, K.; Schaefer, T.; Brüggemann, M.; Grgić, I.; Kubas, A.; Herrmann, H.; Szmigielski, R.

Aqueous-Phase Photo-Oxidation of Selected Green Leaf Volatiles Initiated by OH Radicals: Products and Atmospheric Implications. *Sci. Total Environ.* **2023**, 879, 162622.

(60) Lee, C.-H.; Lee, Y.-Y.; Chang, Y.-C.; Pon, W.-L.; Lee, S.-P.; Wali, N.; Nakazawa, T.; Honda, Y.; Shie, J.-J.; Hsueh, Y.-P. A Carnivorous Mushroom Paralyzes and Kills Nematodes via a Volatile Ketone. *Sci. Adv.* **2024**, 9 (3), No. eade4809.

(61) İlknur Yavasoglu, S.; Wood, M. J.; Alkhaibari, A. M.; Touray, M.; Butt, T. Potential of 3-Octanone as a Lure and Kill Agent for Control of the Brown Garden Snail. *J. Invertebr. Pathol.* **2023**, 198, 107920.

(62) Harvey, R. M.; Zahardis, J.; Petrucci, G. A. Establishing the Contribution of Lawn Mowing to Atmospheric Aerosol Levels in American Suburbs. *Atmos. Chem. Phys.* **2014**, 14 (2), 797–812.

(63) Guenther, A. B.; Jiang, X.; Heald, C. L.; Sakulyanontvittaya, T.; Duhl, T.; Emmons, L. K.; Wang, X. The Model of Emissions of Gases and Aerosols from Nature Version 2.1 (MEGAN2.1): An Extended and Updated Framework for Modeling Biogenic Emissions. *Geosci. Model Dev.* **2012**, 5 (6), 1471–1492.

(64) Winters, A. J.; Adams, M. A.; Bleby, T. M.; Rennenberg, H.; Steigner, D.; Steinbrecher, R.; Kreuzwieser, J. Emissions of Isoprene, Monoterpene and Short-Chain Carbonyl Compounds from Eucalyptus Spp. in Southern Australia. *Atmos. Environ.* **2009**, 43 (19), 3035–3043.

(65) Graus, M.; Schnitzler, J.-P.; Hansel, A.; Cojocariu, C.; Rennenberg, H.; Wisthaler, A.; Kreuzwieser, J. Transient Release of Oxygenated Volatile Organic Compounds during Light-Dark Transitions in Grey Poplar Leaves. *Plant Physiol.* **2004**, 135 (4), 1967–1975.

(66) Isebrands, J. G.; Guenther, A. B.; Harley, P.; Helmig, D.; Klinger, L.; Vierling, L.; Zimmerman, P.; Geron, C. Volatile Organic Compound Emission Rates from Mixed Deciduous and Coniferous Forests in Northern Wisconsin, USA. *Atmos. Environ.* **1999**, 33 (16), 2527–2536.

(67) Jansson, C.; Faiola, C.; Wingler, A.; Zhu, X.-G.; Kravchenko, A.; de Graaff, M.-A.; Ogden, A. J.; Handakumbura, P. P.; Werner, C.; Beckles, D. M. Crops for Carbon Farming. *Front. Plant Sci.* **2021**, 12, 636709.

(68) Peñuelas, J.; Llusà, J. BVOCs: Plant Defense against Climate Warming? *Trends Plant Sci.* **2003**, 8 (3), 105–109.

(69) Rohatyn, S.; Yakir, D.; Rotenberg, E.; Carmel, Y. Limited Climate Change Mitigation Potential through Forestation of the Vast Dryland Regions. *Science* **2022**, 377 (6613), 1436–1439.