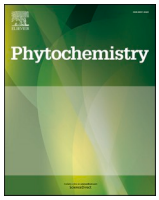
Phytochemistry 221 (2024) 114040

Contents lists available at ScienceDirect

Phytochemistry

journal homepage: www.elsevier.com/locate/phytochem



Drought induces moderate, diverse changes in the odour of grassland species

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ARTICLE INFO

Keywords:
Grassland species
Chemical profile
Plant volatile organic compounds
Drought
Biosynthetically informed distance
Phylogeny
Terpenes

ABSTRACT

Plants react to drought stress with numerous changes including altered emissions of volatile organic compounds (VOC) from leaves, which provide protection against oxidative tissue damage and mediate numerous biotic interactions. Despite the share of grasslands in the terrestrial biosphere, their importance as carbon sinks and their contribution to global biodiversity, little is known about the influence of drought on VOC profiles of grassland species. Using coupled gas chromatography-mass spectrometry, we analysed the odorants emitted by 22 European grassland species exposed to an eight-week-lasting drought treatment (DT; 30% water holding capacity, WHC). We focused on the odorants emitted during the light phase from whole plant shoots in their vegetative stage. Emission rates were standardised to the dry weight of each shoot. Well-watered (WW) plants (70% WHC) served as control. Drought-induced significant changes included an increase in total emission rates of plant VOC in six and a decrease in three species. Diverging effects on the number of emitted VOC (chemical richness) or on the Shannon diversity of the VOC profiles were detected in 13 species. Biosynthetic pathways-targeted analyses revealed 13 species showing drought-induced higher emission rates of VOC from one, two, three, or four major biosynthetic pathways (lipoxygenase, shikimate, mevalonate and methylerythritol phosphate pathway), while six species exhibited reduced emission rates from one or two of these pathways. Similarity trees of odorant profiles and their drought-induced changes based on a biosynthetically informed distance metric did not match species phylogeny. However, a phylogenetic signal was detected for the amount of terpenoids released by the studied species under WW and DT conditions. A comparative analysis of emission rates of single compounds released by WW and DT plants revealed significant VOC profile dissimilarities in four species only. The moderate drought-induced changes in the odorant emissions of grassland species are discussed with respect to their impact on trophic interactions across the food web. (294 words)

1. Introduction

Grasslands constitute about 40 % of the terrestrial biosphere. They are among the most diverse plant communities and play a vital role in carbon sequestration (Bai and Cotrufo, 2022; Buisson et al., 2022; Wilson et al., 2012). Recently increased frequency and intensity of extreme weather events such as prolonged periods of drought in Central Europe are considered as a facet of anthropogenic climate change (Cai et al., 2014, 2015; Coumou and Rahmstorf, 2012) and may eventually result in loss of phylogenetic diversity specifically in grassland communities (Li et al., 2019), in destabilisation of communities (Ives and Carpenter, 2007; Tucker et al., 2018) and in rapid transitions of whole ecosystems (Heger et al., 2019).

Plants invest in volatile organic compounds (VOC) that mediate multiple biotic interactions and protect plant tissues against thermal or oxidative damage. Increased emissions of VOC constitute a typical response of plant leaves to brief drought stress (Loreto and Schnitzler, 2010). However, prolonged water deficit may finally result in a reduced emission of VOC ultimately followed by fatal leaf tissue damage (Haberstroh et al., 2018; Ormeño et al., 2007; Perreca et al., 2020).

Four major biosynthetic routes contribute to most VOC releases, i.e., the lipoxygenase (LOX) pathway leading to fatty acid derivatives

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https://doi.org/10.1016/j.phytochem.2024.114040
Received 2 January 2024; Received in revised form 22 February 2024; Accepted 25 February 2024
Available online 28 February 2024

including green leaf volatiles, the 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway and the mevalonate (MEV) pathway leading to terpenoids, and the shikimate (SHI) pathway producing aromatic compounds (Dudareva et al., 2006). While all four pathways are responsive to and mediate biotic interactions (Dudareva et al., 2006; Junker et al., 2017), effects of heat and drought have predominantly been studied with respect to mono- and sesquiterpene emissions, i.e., products of the MEP and MEV pathways, respectively, which frequently exhibit photoprotective functions (Haberstroh et al., 2018; Loreto and Schnitzler, 2010; Ormeño et al., 2007; Szabó et al., 2020).

Under water deficit, plants may shift carbon partitioning from primary metabolism to the emission of secondary metabolites such as isoprene and monoterpenes from their leaves (Kreuzwieser et al., 2021). However, reallocation of carbon to secondary metabolism is not a universal response to drought. Species without reservoirs of volatile terpenes may alternatively reduce terpene emissions or derive volatile mono- and sesquiterpenes from tri- and tetraterpene stocks. Drought may also induce contrasting trends in terpene emissions of the same species depending on the season, and carbon allocation to drought-induced volatile emissions may be modulated by mycorrhiza (Llusià and Peñuelas, 1998; Llusià et al., 2006; Staudt et al., 2002; Szabó et al., 2020). Water deficit-induced release of the volatile phytohormone methyl jasmonate, a derivative of the LOX pathway, may lead to reduced early drought tolerance in neighbouring plants. Ultimately, this drought-driven interaction may lead to thinning of the vegetation and may be interpreted as competition for water (Jin et al., 2021). Together these reports from a wide range of woody and herbaceous species indicate that diverse responses to non-lethal water deficits must be expected with respect to VOC emissions from vegetative plant organs (Szabó et al., 2020).

In addition to drought, numerous further plant intrinsic and environmental factors impact the emission of VOC from leaves (Dudareva et al., 2006; Junker et al., 2017; Ninkovic et al., 2021). VOC mediate allelopathy and plant competition (Pierik et al., 2012). Their emissions are modified by neighbouring plants (Kigathi et al., 2019), symbiotic and phytopathogenic microorganisms (Ristok et al., 2019). Plant odour can attract (Späthe et al., 2012) or deter herbivores (Zhang and Schlyter, 2004). Insect egg depositions on leaves induce plant volatiles that attracts egg parasitoids (Hilker and Fatouros, 2015). Feeding damage-induced emission of leaf volatiles guides antagonists of herbivores and alerts neighbouring plants to raise their defences (Karban et al., 2014). Thus, biotic interactions of plant species build on VOC emissions in ecological time horizons, but also shape qualitative and quantitative VOC profiles of their vegetative organs from an evolutionary perspective (Buell et al., 2018; Richards et al., 2015; Salazar et al., 2018). The resulting chemical diversity within a plant community may benefit its members (Bustos-Segura et al., 2017; Salazar et al., 2016), stabilise the community (Kerwin et al., 2015) and is expected to be upheld under non-lethal drought stress. Drought also alters floral volatile emissions qualitatively and quantitatively in a biosynthetic pathway- and intensity-dependent manner (Campbell et al., 2018; Rering et al., 2020). Communities of flower visitors may change under drought treatment (Burkle and Runyon, 2016; Glenny et al., 2018). Despite the relevance of drought-induced changes of VOC emissions for individual plants, communities, and interaction networks, drought-induced changes in VOC emissions from vegetative plant organs have to the best of our knowledge only been studied in individual species, small sets of species (Haberstroh et al., 2018; Ormeño et al., 2007; Pagadala Damodaram et al., 2021; Vallat et al., 2005), or in specific families, e.g. the Lamiaceae (Szabó et al., 2020). No study has addressed as yet the question how drought affects the VOC emissions of a broad sample of the member species of a grassland plant community.

To address this gap of knowledge, we investigated the impact of drought on whole-day above-ground, constitutive odorant emissions of 22 plant species, which are common in grassland communities in Central Europe. The grassland species belong to 12 families including three representatives from the Asteraceae and the Fabaceae as well as seven Poaceae (grasses) (Table 1). To reduce the complexity of this investigation, we focussed on VOC emissions during the vegetative phase of all investigated plant species.

In detail, we addressed the following questions by analysing the odorants of plants that had been kept in a greenhouse and exposed to standardised drought conditions for eight weeks. (1) How do VOC patterns emitted by well-watered (WW) and drought-treated (DT) plant species differ with respect to chemical richness and Shannon diversity (Hilker, 2014)? (2) How do total emission rates of VOC and those produced via distinct biosynthetic pathways differ in a WW and DT plant species? (3) Do (dis)similarities of VOC blends and drought-induced emission changes reflect phylogenetic relationships of plants? (4) How do similarities of drought-induced VOC profiles based on biosynthetically informed distances (Junker, 2018) relate to similarities of drought-induced changes in morphological traits analysed by Lozano et al. (2020)? By using the same experimental setup as we used here, Lozano et al. (2020) studied the impact of drought on size and biomass of the same grassland species. They found that drought triggered a reduction in shoot biomass, specific leaf area, and an increase in leaf dry matter content in most species. (5) Finally, in addition to entire blends or biosynthetic pathways, we analysed the emission rates of individual VOC in DT and WW plants of each species and addressed the question of which compounds contribute to blend dissimilarities. All odorant emission rates were calculated per hour and standardised to whole shoot dry weight.

2. Results and discussion

Odorants emitted above ground from 22 grassland plant species in their vegetative, non-flowering stage were collected for 16 h (12 h light; 4 h darkness; Supplementary Fig. S1) from DT and WW plants (each treatment $N = 5$ per species). This sampling period warranted a coverage of VOC emissions widely independent of species- and compound-specific diurnal emission timelines (Niinemets et al., 2004). The headspace samples were analysed by coupled gas chromatography – mass spectrometry (GC-MS).

The analyses revealed rich patterns of VOC, of which 78 were included in our statistical analysis. Among these, we identified 14 green leaf volatiles and other LOX pathway derivatives, eight aromatic products of the SHI pathway, 21 products of the MEP pathway (17 monoterpenes, one monoterpene derivative, three carotene derivatives), and 29 products of the MEV pathway (27 sesquiterpenes, one sesquiterpene derivative, one triterpene) (Table 2). Furthermore, two aliphatic hydrocarbons (non-terpenoid) as well as two ketones, one cyclic alcohol and one furan derivative met the quantitative criterion to be included in

Table 1
List of European grassland species, which were analysed for their changes in emission of plant volatile organic compounds (VOC) in response to an eight-week drought period.

Plant family	Plant species
Asteraceae	*Achillea millefolium* L., *Artemisia campestris* ssp. *campestris* L., *Hieracium pilosella* L.
Apiaceae	*Daucus carota* L.
Brassicaceae	*Berteroa incana* L.
Caryophyllaceae	*Silene vulgaris* (Moench) Garcke
Fabaceae	*Trifolium repens* L., *Vicia cracca* L., *Medicago lupulina* L.
Guttiferae	*Hypericum perforatum* L.
Plantaginaceae	*Plantago lanceolata* L.
Plumbaginaceae	*Armeria maritima* ssp. *elongata* (Hoffm.) Bonnier
Poaceae	*Arrhenatherum elatius* (L.) P.Beauv. ex J.Presl & C.Presl, *Holcus lanatus* L., *Poa angustifolia* L., *Anthoxanthum odoratum* L., *Lolium perenne* L., *Festuca rubra* L., *Dactylis glomerata* L.
Ranunculaceae	*Ranunculus acris* L.
Rosaceae	*Potentilla argentea* L.
Rubiaceae	*Galium verum* L.

Table 2
Plant volatile organic compounds (VOC) included in the analyses. Bold letters indicate compounds identified by comparison of mass spectra and RI to authentic standards; * indicate chiral structures not resolved to their enantiomers. PW: Biosynthetic pathway or other grouping variable (see text body). MW: Molecular weight. MP: *m/z* values of $[M]^+$; ( ) indicate the highest *m/z* value detected in the absence of the actual $[M]^+$. BP: Base peak; ( ) designate *m/z* values close to identical to the actual BP. RI: Retention time index based on n-alkane (C7–C40) reference runs. Lib - RI: values from reference substances (bold) or National Institute of Standards (NIST) MS libraries; ( ) indicate estimated values (NIST MS library).

Compound	PW	CAS	MW	MP	BP	RI	Lib - RI
hexanal	LOX	66-25-1	100	100	56(44)	801	**798**
(*E*)-2-hexenal	LOX	6728-26-3	98	98	41	855	**853**
(*Z*)-3-hexen-1-ol	LOX	928-96-1	100	100	67	859	**855**
1-octen-3-one	LOX	4312-99-6	126	126	55	979	978–988
1-octen-3-ol*	LOX	3391-86-4	128	128	57	980	**979**
hexanoic acid	LOX	142-62-1	116	(87)	60	985	**988**
3-octanone	LOX	106-68-3	128	128	43	988	979–992
3-octanol*	LOX	589-98-0	130	(112)	59	997	**996**
(*E*,*Z*)-2,4-heptadienal	LOX	881395	110	110	81	997	996
(*Z*)-3-hexenyl acetate	LOX	3681-71-8	142	(82)	67	1008	**1008**
(*E*,*E*)-2,4-heptadienal	LOX	4313-03-5	110	110	81	1013	1011
(*E*)-3-octen-2-one	LOX	18402-82-9	126	126	55	1039	**1038**
5-ethyl-2(5H)-furanone	LOX	2407-43-4	112	112	83	1039	n.a.
1-octen-3-yl-acetate	LOX	2442-10-6	170	170	43	1117	1105–16
benzaldehyde	SHI	100-52-7	106	106	77	962	**962**
o-methyl anisole	SHI	578-88-5	122	122	122	1009	1009–13
benzyl alcohol	SHI	100-51-6	108	108	79	1034	**1035**
2-phenylethanol	SHI	60-12-8	122	122	91	1117	**1113**
1,3-dimethoxybenzene	SHI	151-10-0	138	138	138	1169	1158–81
benzyl isovalerate	SHI	103-38-8	192	192	91	1397	1385–96
coumarin	SHI	91-64-5	146	146	118	1445	1432–56
ethyl p-ethoxybenzoate[b]	SHI	23676-09-7	194	194	121	1529	1521–37
2-methyloctane	HC	3221-61-2	128	128	43	862	858–868
nonane	HC	111-84-2	128	128	43(57)	900	**900**
4-methyl-3-pentene-2-one	MEP	141-79-7	98	98	83	803	800–804
α-pinene	MEP	80-56-8	136	136	93	932	**932**
sabinene*	MEP	3387-41-5	136	136	93	973	**973**
β-pinene	MEP	18172-67-3	136	136	93	976	**975**
6-methyl-5-hepten-2-one	MEP	110-93-0	126	126	43	988	**988**
β-myrcene	MEP	123-35-3	136	136	93	993	**990**
3-carene	MEP	13466-78-9	136	136	93	1010	**1009**
p-cymene	MEP	99-87-6	134	134	119	1025	**1024**
limonene*	MEP	5989-27-5	136	136	68	1029	1028–31
n.i. monoterpene 145	MEP		136	136	93	1029	
eucalyptol (syn. cineol)	MEP	470-82-6	154	154	43	1031	**1031**
(*Z*)-β-ocimene	MEP	3338-55-4	136	136	93	1039	**1038**
n.i. (*E*)-β-ocimene or (*Z*)-α-oximene	MEP		136	136	93	1049	1046–51
borneol*	MEP	10383-78-1	154	154	95	1168	**1166**
p-mentha-1,5-dien-8-ol	MEP	1686-20-0	152	(137)	94	1169	1160–81
n.i. monoterpene	MEP		152	152	119	1169	1227
carveol	MEP	99-48-9	152	152	119	1209	1217–20
(3*E*,5*E*)-2,6-dimethyl-(3,5,7)-octatriene-2-ol	MEP	NIST# 141118	152	152	43	1209	1209
β-damascone (carotenoid derivative)	MEP	85949-43-5	192	192	177	1418	1409–19
(*E*)-β-ionone (carotenoid derivative)	MEP	79-77-6	192	192	177	1490	**1489**
n.i. ionone epoxide (carotenoid derivative)	MEP	NIST# 192140	208	(135)	123	1490	1488
(*E*)-4,8-dimethylnona-1,3,7-triene	MEV	19945-61-0	150	150	69	1117	1117
(−)-α-copaene*	MEV	3856-25-5	204	204	119 (161)	1381	**1385**
β-cubebene	MEV	13744-15-5	204	204	161	1392	1388–98
β-elemene*	MEV	515-13-9	204	204	81 (93)	1397	1389–99
n.i. sesquiterpene 159	MEV		204	204	119 (105)	1419	
n.i. sesquiterpene 61	MEV		204	204	161	1420	1418–28
(−)-α-cedrene	MEV	469-61-4	204	204	119	1420	**1424**
α-bergamotene	MEV		204	204	93 (119)	1420	1414–36
n.i. sesquiterpene 66	MEV		204	204	147	1424	
n.i. sesquiterpene 67	MEV		204	204	161	1424	
(−)-β-caryophyllene	MEV	87-44-5	204	204	93 (133)	1426	**1423**
(+)-β-cedrene	MEV	546-28-1	204	204	161	1428	1417–28
β-sesquisabinene*	MEV	58319-04-3	204	204	69	1447	1444–67
guaia-6,9-diene*	MEV	36577-33-0	204	204	105	1450	(1440)
(*E*)-β-farnesene	MEV	18794-84-8	204	204	69	1459	1453–61
n.i. sesquiterpene(s) 150[a]	MEV		204	204	161/91	1460	
α-humulene	MEV	6753-98-6	204	204	93	1461	**1460**
4a,8-dimethyl-2-(prop-1-en-2-yl)- 1,2,3,4,4a,5,6,7-octahydronaphthalene	MEV	103827-22-1	204	204	189	1482	1485–91
γ-muurolene	MEV	30021-74-0	204	204	161	1482	1477–88
β-acoradiene*	MEV	24048-44-0	204	204	119	1482	1470–83
n.i. sesquiterpene 142	MEV	5989-08-2	204	204	119	1483	1490–10
D-germacrene	MEV	23986-74-5	204	204	161	1488	1460–91
n.i. sesquiterpene 34	MEV	NIST# 104201	204	204	92	1491	1478–84
n.i. (±)-cadinene* or δ-selinene	MEV		204	204	161 (189)	1497	1472–15
α-selinene*	MEV	473-13-2	204	204	189	1502	1480–15

(*continued on next page*)

Table 2 (*continued*)

Compound	PW	CAS	MW	MP	BP	RI	Lib - RI
elixene	MEV	3242-08-8	204	204	121	1503	1492–11
α-muurolene*	MEV	31983-22-9	204	204	105	1506	1497–19
cadina-1(10),4-diene	MEV	16729-01-4	204	204	161	1530	1498–40
squalene	MEV	111-02-4	410	410	69	2839	2833–47
4-cyclopentene-1,3-dione	div	930-60-9	96	96	96	884	882
2-pentylfuran	div	3777-69-3	138	138	81	993	987–996
2,2,6-trimethyl-cyclohexanone	div	2408-37-9	140	140	82	1036	1035–36
2,6-dimethyl-cyclohexanol*	div	5337-72-4	128	128	71	1109	1110–14

[a] BP values separated by "/" indicate unresolved coelution of two sesquiterpenes.
[b] Ethyl p-ethoxybenzoate has consistently been identified in a subset of species (Fig. 2). It has been identified from other natural sources (Mann et al., 2017; Oku et al., 2015; Ozcan and Chalchat, 2007). However, we caution that this compound is also known as plastic additive (Sait et al., 2021).

the analyses (see section 3.5.). The latter compounds might have diverse biosynthetic origins (Charpentier et al., 2012); therefore, they were not assigned to any of the aforementioned pathways. Unless stated otherwise, all drought-induced changes in VOC emission patterns presented in the following sections were determined by analysing normalised relative index of interaction intensity (RII) values (Armas et al., 2004), see section 3.10.

2.1. Shannon diversity and chemical richness

Drought led to a significant increase of the Shannon diversity H′ of the investigated chemical profiles in five species, whereas another five species displayed a significant decrease (Fig. 1). Across species, the Shannon diversity H' ranged between 2.3 ± 0.50 to 3.4 ± 0.10 (mean ± s.d.) in WW plants, and between 2.3 ± 0.72 to 3.3 ± 0.18 in DT plant species (Supplementary Table S1, first chart).

Chemical richness, i.e., the number of VOC detected, was compared between WW and DT plants and between plant species within each treatment group. Chemical richness varied greatly across the analysed species. Within the group of WW plants, the richness ranged from 13.0 ± 2.00 (mean ± s.d.) compounds in *Ranunculus acris* L. to 42.4 ± 3.85 compounds in *Hypericum perforatum* L. Within the group of DT plants, the richness values ranged from 15.2 ± 7.95 compounds in *Hieracium pilosella* L. to 37.6 ± 8.62 in *H. perforatum*. Drought significantly increased the compound richness in four species, while the opposing effect was observed in six species (Fig. 1, Supplementary Table S1, second chart).

Chemical diversity has evolved as a benefit for plants when coping with herbivory (Becerra et al., 2009; Whitehead et al., 2021), while specific compounds, mostly terpenes, also provide protection against physical stressors such as drought or irradiation (Loreto and Schnitzler, 2010). Plants exposed to drought might respond by redirecting their biosynthetic machinery to compounds providing protection against the dominant stressor. The enhanced biosynthesis of a few protective chemicals might be at the expense of other chemicals, thus resulting in reduced chemical richness or lowered Shannon diversity. Alternatively, plants could maintain or enhance chemical diversity under non-lethal abiotic stress as an adaptation to prospective biotic, often antagonistic interactions, which are ubiquitous in highly biodiverse ecosystems such as grasslands (Wilson et al., 2012).

In our experiment drought triggered negative effects on chemical richness and Shannon diversity of the odorant profiles in only five and six out of 22 species, respectively. Thus, maintaining and enhancing chemical richness and Shannon diversity in response to non-lethal drought, appeared as the dominant but not exclusive strategy in grassland species. This result is in line with benefits of chemical diversity for individual plants and plant populations experiencing biotic interactions (Bustos-Segura et al., 2017; Kerwin et al., 2015; Richards et al., 2015; Salazar et al., 2016, 2018).

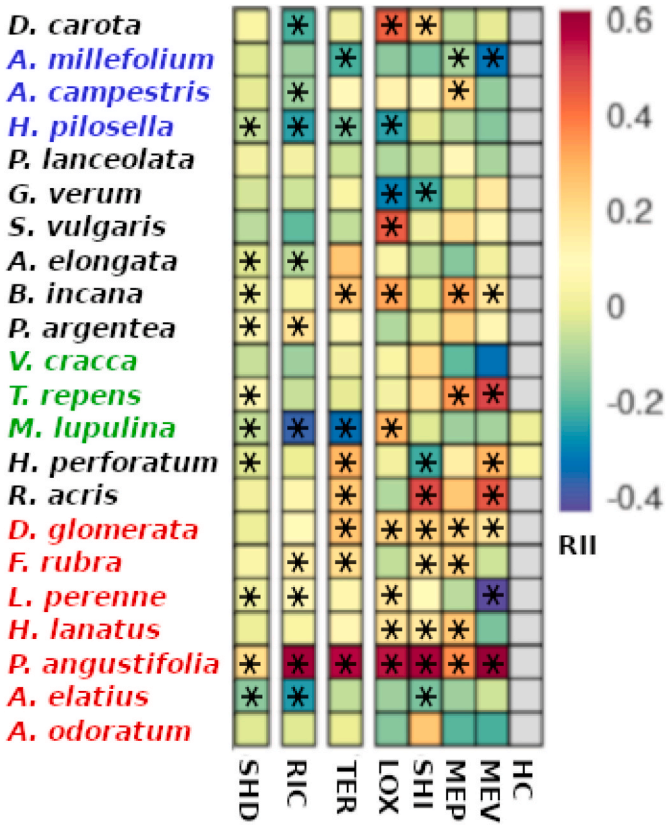

Fig. 1. Drought-induced changes in plant odorant profiles. Changes in chemical diversity calculated by the Shannon Diversity Index (SHD), in chemical richness, i.e. the number of compounds detected (RIC), and in total emission rates (TER) of all analysed VOC. In addition to changes in total emission rates, also changes in emission rates of VOC produced via the lipoxygenease pathway (LOX), the shikimate pathway (SHI), and the terpenoid pathways MEP and MEV as well as hydrocarbons (HC) are given. Full plant species names are given in Table 1; colours indicate plant families: Blue - Asteraceae; green - Fabaceae; red - Poaceae; black - all other species. Normalised changes are indicated by colour-coded positive and negative RII (relative interaction intensity index) values, which indicate increased and decreased variables when comparing DT to WW plants. Grey fields indicate the absence of a compound class. An * marks significant changes with $P < 0.05$ (one sample *t*-tests); see text body and Supplementary Table S1 for statistical details. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

2.2. Normalised emission rates

2.2.1. Total VOC emission rates

Total aboveground VOC emission rates of the 22 plant species were determined by summarising the emissions of all analysed compounds

normalised to shoot dry weight. The total emission rates varied over a wide range across the analysed species. In WW plants, they ranged from 0.23 ± 0.112 ng mg^{-1} h^{-1} (mean ± s.d.) in *Poa angustifolia* L. to 1.66 ± 0.374 ng mg^{-1} h^{-1}in *H. perforatum*. The DT plants showed a similar range of total emission rates (lowest: 0.25 ± 0.075 ng mg^{-1} h^{-1} in *Festuca rubra* L.; highest: 3.40 ± 3.044 ng mg^{-1} h^{-1} in *H. perforatum*) (Supplementary Table S1, chart 3).

In six species, total volatile emission rates significantly increased in DT compared to WW plants. A significant decrease was observed in three species (Fig. 1). In absolute values, drought-treated *P. angustifolia* and *H. perforatum* showed an about three- and twofold higher total VOC emission rate, respectively. *Dactylus glomerata* L. and *Berteroa incana* L. showed an about 1.6-fold higher total emission rate than conspecific WW plants. In WW plants, a pronounced increase in total VOC emission rates compared to drought treatment by factor 1.6 was observed in *H. pilosella* (Supplementary Table S1, chart 3). In four out of nine species that displayed significant changes in total emission rates both measures of chemical diversity remained unaffected. Conversely, in seven out of 13 species showing significant changes in one or both measures of chemical diversity drought had no impact on total emission rates. Thus, significant changes in total emission rates did not correspond to significant changes in both measures of chemical diversity. In contrast, significant changes in total emission rates were always associated with significant changes of VOC emissions from one or more of the investigated biosynthetic pathways. However, eight species displayed significantly altered VOC emission from one, two, or three pathways without resulting in a significant change of total emission rates (Fig. 1).

Effects of drought on the summarised emission of VOC have particularly been reported for the MEP and MEV pathway (e.g. Haberstroh et al., 2018; Loreto and Schnitzler, 2010; Ormeño et al., 2007; Szabó et al., 2020). Studies calculating total emission rates as sum of emissions from all relevant pathways are scarce in drought-exposed plants. When exposing two-year-old seedlings from four European tree species to drought, Fitzky et al. (2023) observed a significant decrease of VOC total emissions rates only in *Quercus robur*, while results were not significant in the other tested species *Carpinus betulus*, *Fagus sylvatica*, and *Betula pendula*. In another tree species, *Pinus massoniana*, drought led to reduced total VOC emission (Huang et al., 2023). Mixed findings with respect to total emission rates were also reported from drought-exposed flowers. One out of three brassicaceous species displayed enhanced floral VOC total emission rates after drought treatment (Höfer et al., 2022), and total emission rates increased with the duration of drought in flowers from *Ipomopsis aggregata*, a subalpine herb (Campbell et al., 2018). Similar to our results, in all above-mentioned studies, the secondary metabolite pathways LOX, SHI, MEP, and MEV contributed to the observed changes of total emission rates in species-specific combinations.

2.2.2. Emission rates of VOC from different biosynthesis pathways

We addressed the question whether emission rates of VOC produced by a certain biosynthesis pathway are differentially affected in WW and DT plants. Pathway-specific emission rates of VOC produced via the LOX, SHI, MEP and MEV pathway were separately compared between DT and WW plants of each species (Fig. 1). We did not observe a universal increase or decrease of any specific biosynthetic class of VOCs across all plant species.

Significant increases in LOX pathway-specific emission rates between DT and WW plants were detected in eight species. *Daucus carota* L. showed an almost fivefold increase, *P. angustifolia*, *B. incana*, *Medicago lupulina* L., and *Silene vulgaris* (Moench) Garcke showed an about twofold increase of absolute emission of VOC produced via the LOX pathway when exposed to drought. A significant decrease of LOX pathway-derived emissions was observed in *H. pilosella* and *Galium verum* L. (Fig. 1, Supplementary Table S1, chart 4).

Our finding that only few species showed enhanced emission of VOC biosynthesized via the LOX pathway is surprising since this pathway is long known to respond to drought by upregulation of *LOX* (Avramova et al., 2015; Bell and Mullet, 1991). Correspondingly, plant exposure to drought was shown to result in increased emissions of products of the LOX pathway in e.g., apple trees and citrus species (Vallat et al., 2005; Vieira et al., 2016). Furthermore, high drought-induced emission rates of LOX pathway derivatives were detected in plant species that responded to drought also by leaf senescence and morphological tissue changes (Capitani et al., 2009; Jardine et al., 2015). In our experiment, we did not observe signs of leaf senescence or wilting in DT plants of the above-mentioned species with increased emission rates of LOX-derived VOC.

Emissions of aromatic compounds produced via the SHI pathway were significantly increased in five and decreased in three species in DT plants. Pronounced absolute increases in DT plants by a factor of about 3 and by a factor of 1.4 were observed in *P. angustifolia* and *H. lanatus*, respectively. None of the other plant species showed a change in SHI-derived VOC emission rates in response to drought (Fig. 1, Supplementary Table S1, chart 5). In contrast, drought is well known to induce the production of a wide range of SHI pathway derivatives, which have anti-oxidative activities (Kumar et al., 2021; Liu et al., 2019; Tattini et al., 2015). Many of these drought-induced SHI pathway derivatives are non-volatile compounds. Among stress-inducible, volatile SHI pathway products, methyl salicylate (MeSA) has intensively been studied. Its emission is inducible not only by drought, but also by phytopathogen infection of plants or infestation by herbivorous arthropods (De Boer and Dicke, 2004; Jin et al., 2021; Shulaev et al., 1997). MeSA is well known to be involved in plant defences against herbivores (Ninkovic et al., 2021 and references therein), and to mediate intra- and inter-plant signalling for protection against phytopathogens as well as herbivores (e.g. Heil and Ton, 2008; Pickett et al., 2003). In our study, however, MeSA emissions of the analysed plant species were below the threshold for inclusion in the statistical analysis (compare section 3.5.).

The MEP and MEV pathway are the major biosynthesis routes for production of monoterpenes and sesquiterpenes, respectively (Tholl, 2015). The two pathways interact when the plastidially localized MEP pathway provides isopentenyl diphosphate, a terpene precursor, which enters the cytosol, where the MEV pathway is localized (Dudareva et al., 2005). In our study, drought induced a significant increase of monoterpene emission rates in seven and a significant reduction in one species (Fig. 1). Absolute emission rates increased twofold in *P. angustifolia* as well as in *B. incana* (Supplementary Table S1, chart 6). Drought induced significantly higher and lower sesquiterpene emission rates in six and two species, respectively. Pronounced absolute increases were observed in *P. angustifolia* and *Trifoliium repens* L. by about factor 3, in *H. perforatum* by factor 2.4, and in *D. glomerata* by about factor 1.4. Five species analysed here showed similar significant drought-induced changes in total emission rates of monoterpenes and sesquiterpenes (Fig. 1, Supplementary Table S1, charts 6 and 7). Studies of other plant species (including trees and shrubs) revealed very species- and season-specific effects of drought on terpene emissions. While some species showed increased terpene emission rates, others did not change their terpene emissions or even reduced them when experiencing drought during a certain season (Llusià et al., 2006; Ormeño et al., 2007; Staudt et al., 2002).

In addition to the summarised emission rates of all compounds originating from the same biosynthetic pathway (Fig. 1), we also analysed how the emission rate of each VOC changed in response to drought (Fig. 2). Drought-induced changes were detected for many compounds of the LOX, SHI, MEP and MEV pathways. An overview of the emission rate of each volatile compound released by each plant species is given in Supplementary Fig. S2 separately for WW and DT plants. The highly scattered colour pattern in Fig. 2 and Supplementary Fig. S2 visualises very species-specific VOC patterns and responses to drought.

Taken together, the results showed that 17 of the investigated 22 grassland species displayed pathway-specific drought-induced VOC emission rate changes but only two of the analysed grassland species

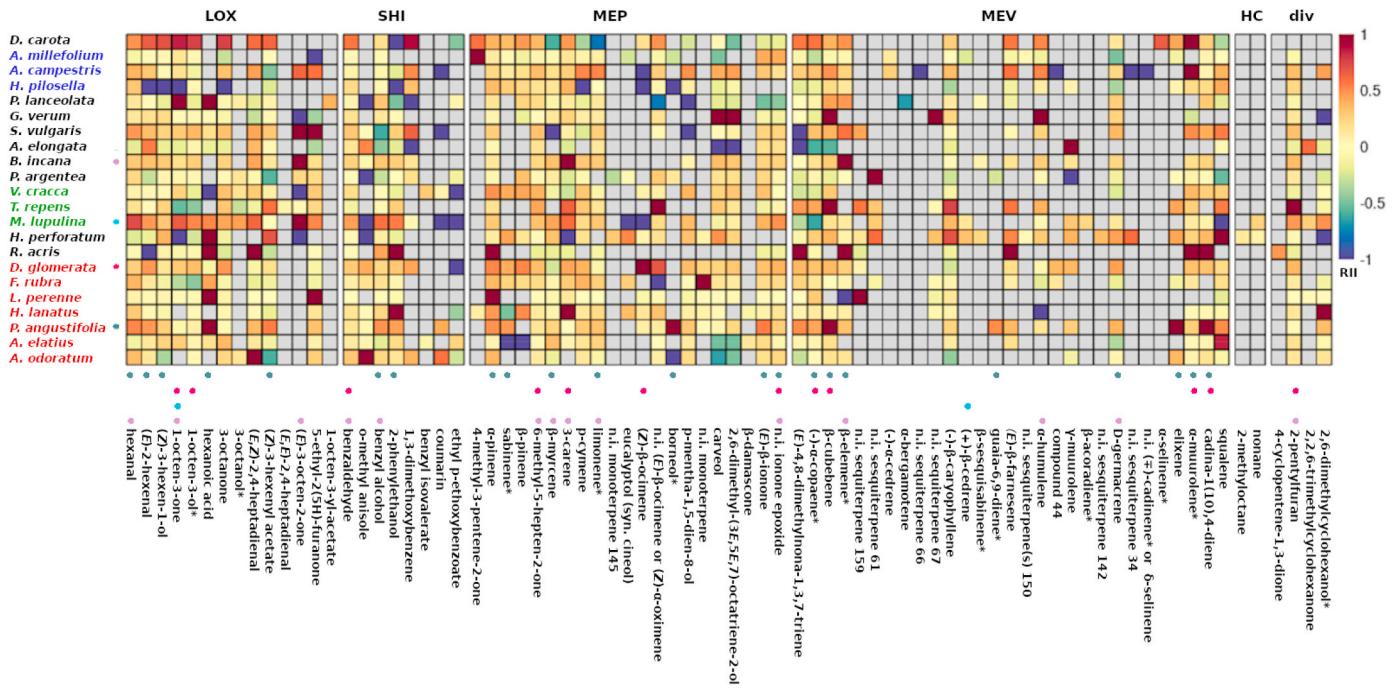

Fig. 2. Drought-induced changes in emission of individual plant volatile organic compounds (VOC). Full plant species names are given in Table 1; colours indicate plant families: Blue - Asteraceae; green - Fabaceae; red - Poaceae; black - all other species. Colour-coded RII (relative interaction intensity index) values represent normalised emission rate differences between treatments. Positive and negative values indicate increases and decreases when comparing DT to WW plants. Chemicals are grouped by their biosynthetic origin or their class and ordered from left to right by ascending retention time. LOX: lipoxygenase pathway, SHI: shikimate pathway; MEP and MEV: terpenoid biosynthesis pathways; HC: aliphatic non-terpenoid hydrocarbons; div: compounds of other biosynthetic origin. Grey fields indicate compounds below detection limit in the respective species. Coloured dots indicate compounds, which contribute significantly to treatment-specific VOC profiles (ANOSIM, SIMPER) of plant species in Table 3. Light pink dot: *Berteroa incana;* turquoise dot: *Medicago lupulina,* intense pink dot: *Dactylus glomerata;* green dot: *Poa angustifolia.* Compare also Supplementary Fig. S2.

responded to drought by significant increases in VOC emission rates from all pathways. Five species displayed no significant change at all (Fig. 1). Still, it is noteworthy that significantly augmented emission rates of compounds produced via one, two, three or all main pathways were observed in 11 of the 22 species studied. Two and four species displayed mixed or negative pathway-specific changes of emission rates, respectively (Fig. 1). The analysis of the drought-induced emission rates of each VOC (Fig. 2) revealed that none of the studied grassland species shared the same set of compounds with any other species. Neither did any species display a uniform drought-driven trend for all or most compounds emitted by that species, nor did any compound by trend display similar responses to drought across all species.

2.3. (Dis)similarities of VOC profiles in relation to plant phylogeny

In addition to the comparison of VOC profiles in WW and DT plants, we addressed the question whether the odorant profiles of the 22 analysed plant species are more similar the closer related the species are. Furthermore, we analysed whether drought-induced changes in VOC profiles reflect the plants' phylogeny. These questions were addressed by applying analyses based on "biosynthetically informed distances" and by calculating phylogenetic signal values (Pagel's λ). We applied the biosynthetically informed distance as a measure of VOC blend similarity, which takes the emission rates of each of the analysed compounds and the proportion of shared biosynthetic routes into account (Junker, 2018; Junker et al., 2017).

First, we studied whether the biosynthetic distances between plant odorant profiles characterized by the emission rate of each VOC are related to the plant's phylogeny. Based on the results of the emission rate of each VOC (Fig. S2), we calculated the biosynthetic distances between odorant profiles of the 22 plant species. We sorted the calculated distances by hierarchical clustering in a tree and compared the calculated tree to the plants' phylogenetic relationships. The resulting tanglegram (Supplementary Fig. S3) highlights that odorant emission patterns of both WW and DT plants were independent of phylogeny.

Furthermore, when analysing whether the biosynthetic distances between plant VOC profiles characterized by drought-induced changes are related to the plant's phylogeny, a similar tanglegram emerged (Fig. 3). Similarities of drought-induced changes of odorant profiles of the investigated plants also did not correspond to phylogenetic relationships. Results were similar when applying Bray-Curtis distances instead of biosynthetically informed distances (results not shown). Hence, no indications were detected that the similarity of VOC profiles resembles the phylogenetic relatedness of the plant species, neither when considering odorants of WW nor of DT plants. Furthermore, no indication was found that drought-induced changes in VOC profiles are related to plant phylogeny.

To further elucidate whether similarities of the determined plant VOC traits might be attributed to evolutionary conservatism, we tested for phylogenetic signals. Significant Pagel's λ values were neither detected for drought-induced changes in total emission rates per compound class, nor for drought-induced changes in chemical richness or Shannon diversity of VOC emissions of each compound class (Fig. 4, Supplementary Table S2).

However, significant phylogenetic signals with a Pagel's λ value ~1 were found for summarised emission rates of compounds from the terpenoid pathways, i.e., from both the MEP and MEV pathway in WW plants and from the MEV pathway only in DT plant (Fig. 4, Supplementary Table S2). These findings suggest that the analysed grassland species display rather ancestral, conserved levels of investment into terpenoid synthesis. Interestingly, VOC profiles of only the DT plants also showed significant phylogenetic signals for the chemical richness and the Shannon diversity of compounds produced via the SHI pathway. This finding indicates that grassland species show conserved responses

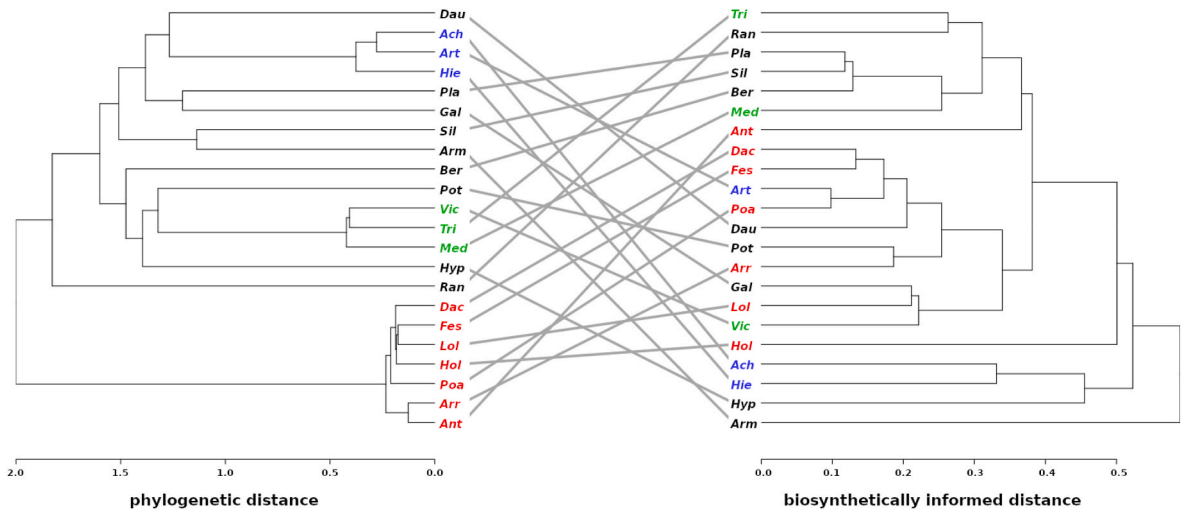

Fig. 3. Tanglegram opposing the phylogenetic tree of the studied species to a dendrogram of drought-induced VOC profile changes in these species. Full plant species names are given in Table 1; colours indicate plant families: Blue - Asteraceae; green - Fabaceae; red - Poaceae; black - all other species. Grey lines in the central area link a species' position on each side of the tanglegram.
All phylogenetic clades or species on the same branches of the phylogenetic tree (left) became dispersed on the dendrodogram of VOC response profiles to drought (right). Distances between nodes represent evolutionary time since species separation..

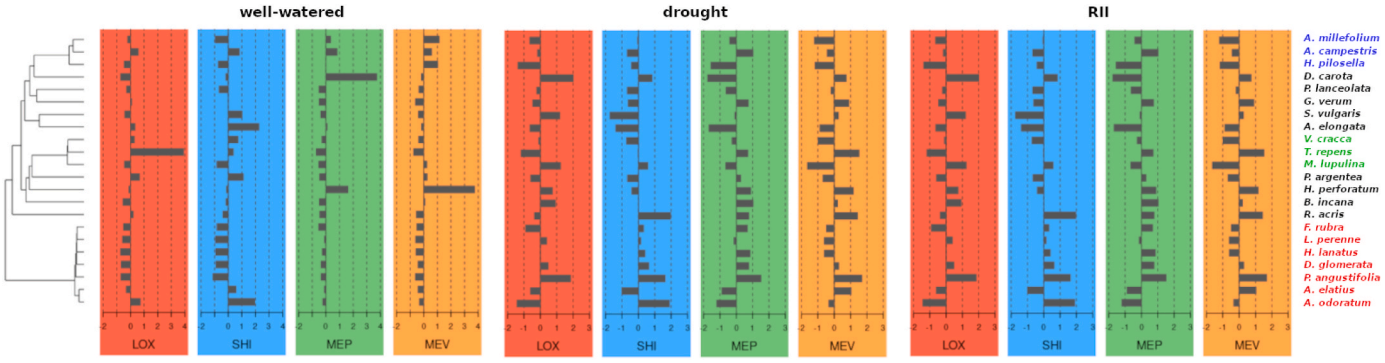

Fig. 4. Scaled and centred representation of emission rates of 22 grassland species summarised by pathway for WW (left) and DT plants (centre). Scaled and centred RII values (relative interaction intensity index; right) represent normalised emission rate changes comparing DT to WW plants. Full plant species names are given in Table 1; colours indicate plant families: Blue - Asteraceae; green - Fabaceae; red - Poaceae; black - all other species; the phylogenetic tree is displayed on the left side. Significant phylogenetic signals with a Pagel's λ value ~1 were found for both terpenoid pathways (MEP and MEV) in WW plants and for the MEV pathway in DT plants (**: $P = 0.001$, Supplementary Table S2).

to drought with respect to the emission of SHI pathway derived VOC.

Odorant profiles of both numerous herbaceous and perennial plant species have been investigated with respect to their phylogenetic signals. These studies show that the detection of a phylogenetic signal in plant VOC blends is strongly dependent on the studied species and the analysed emission trait. When searching for a phylogenetic signal in the diversity of VOC emissions or in total emission rates, no uniform pattern was detected so far. For example, the chemical diversity of terpene blends of more than 200 tropical tree species (Courtois et al., 2016) and the total emission rates of terpenes from 70 Hawaiian woody species showed a strong phylogenetic signal (Llusià et al., 2010). In contrast, the total terpene emission patterns of flowers of 52 forb species in a subalpine meadow in Montana, USA, showed no phylogenetic signal (Burkle et al., 2020). The search for a phylogenetic signal in the emission of a single volatile compound, i.e. linalool, across 48 plant species belonging to 45 genera was negative in the Lamiaceae (Buell et al., 2018). The search for phylogenetic signals in VOC blends might provide such different results because the emission of VOC is not only dependent on the plant's intrinsic, evolutionary evolved abilities, but also on numerous abiotic and biotic conditions (Grote et al., 2019; Peñuelas and Staudt, 2010).

Our study of the relatedness of plant VOC emissions with the plant's phylogeny further shows how much the results also depend on the type of analysis. The biosynthetic distance measure indicates no relationships between the overall biosynthetic activities in odorant production and plant phylogeny, regardless of considering WW or DT plants or drought-induced changes in plant odorant emissions. However, when specifically considering biosynthesis pathways and analysing these for phylogenetic signals, it turned out that the VOC profiles show some evolutionary conservatism with respect to total terpene emission (both in WW and DT plants) and with respect to SHI pathway emission when responding to drought (Fig. 4, Supplementary Table S2). Lozano et al. (2020) analysed how drought-induced changes in morphological parameters of the same plant species as those studied here relate to plant phylogeny. Their analyses also revealed that a link between plant phylogenetic distance and drought-induced changes in plant morphological parameters is highly dependent on the plant trait considered.

2.4. Drought-induced VOC profiles in relation to drought-induced plant morphological traits

Even though drought-induced changes in shoot morphological

parameters showed only a weak link to plant phylogeny, the morphological plant responses to drought might still reflect plant VOC profile responses to drought. Therefore, we addressed the question how drought-induced changes in VOC profiles relate to drought-induced changes in shoot biomass, leaf dry matter content (LDMC) and the specific leaf area (SLA) measured by Lozano et al. (2020) (Supplementary Table S1). A tanglegram comparing clusters of drought-induced changes in morphological traits and biomass with drought-induced changes in chemical profiles revealed that morphological trait-based response clusters were independent of drought-induced changes in VOC profiles and vice versa (Supplementary Fig. S4).

2.5. Drought-specific VOC profiles

In addition to the analyses described above, we also compared emission rates of all compounds emitted by DT and WW plants irrespective of their biosynthetic pathway.

We first conducted an analysis of similarity (ANOSIM) that was based on Bray-Curtis distances, i.e., treating each chemical as an independent information entity irrespective of biosynthetic origin. This ANOSIM tested whether distances between samples belonging to different treatment groups are greater (less similar) than within groups. The ANOSIM was followed by an assignment of percentage values (SIMPER) indicating the degree to which each compound contributes to the dissimilarity of VOC profiles under each treatment within species (Fig. 2, Table 3, Supplementary Table S3).

Significantly distinct chemical profiles of WW and DT plants were identified in *B. incana*, *M. lupulina*, *D. glomerata*, and *P. angustifolia*. Except for *M. lupulina,* more than a dozen products from all major pathways contributed to VOC blend dissimilarity in the DT and WT plants of these species (Table 3). In *M. lupulina*, only a single compound each of the LOX- and the MEV pathway significantly contributed to the drought-specific blend composition. Remarkably, all compounds that significantly contributed to dissimilar VOC blends were emitted at enhanced rates in DT compared to WW plants. We studied only $N = 5$ plants per species and treatment, and low replicate numbers may conceal significant results. However, the chemical profiles of DT und WW plants were close to significantly different in only two other species (*D. carota* and *Artemisia campestris* L.; ANOSIM p-values below 0.1, Supplementary Table S3). The majority of the investigated grassland species did not even differ by trend in their odorant blend composition in DT and WT plants. Thus, resilience to drought-induced change in chemical profiles might be a characteristic in the investigated grassland species.

Our results indicate that drought can species-specifically affect distinct steps within the LOX, SHI, MEV and MEP pathway and thereby induce a change in emission rates of specific compounds. A shift in the quantitative emission rate of a single VOC results in a change of quantitative ratios of VOC within a blend, which may significantly affect ecological interactions of plants with the second and third trophic level. The ratios of VOC within a blend are well known to be crucial for olfactory-guided host foraging behaviour of many herbivorous and carnivorous insects (Beyaert and Hilker, 2014; Bruce and Pickett, 2011).

2.6. Concluding remarks

Our study showed that the applied drought treatment had overall moderate effects on VOC emissions in the grassland species studied here. Drought affected each analysed trait, i.e. chemical richness, Shannon-diversity, total emission rate, pathway specific emission rates, and VOC profiles in a minority of the investigated species. Trait values increased or decreased species specifically in response to drought, while drought had not detectable effects at all on VOC emissions from three species. This finding is surprising at a first glance because morphological above-ground plant traits like specific leaf area and shoot biomass of most of these species were significantly reduced by the drought treatment (Lozano et al., 2020). These drought-induced changes in plant morphology were barely reflected by changes in VOC emissions. In the following we will address three factors that likely contributed to these findings.

First, the natural soil microbiome might have limited drought-induced changes in aboveground VOC emissions of the studied

Table 3
Compounds contributing to drought-specific VOC profiles.

Species R-value[a] P-value[b]	Compound SIMPER % contribution to VOC blend dissimilarity; P value				
B. incana	3-carene	1-octen-3-one 3.5; 0.018	D-germacrene 3.3; 0.008	2-pentylfuran 3.2; 0.018	β-myrcene
R = 0.404	3.8; 0.008				3.2; 0.018
P = 0.0149	benzyl alcohol 3.1; 0.033	α-humulene 2.8; 0.034	hexanal	n.i. ionone epoxide	benzaldehyde 2.4; 0.008
			2.7; 0.046	2.5; 0.049	
	limonene	(*E*)-3-octen-2-one	6-methyl-5-hepten-2-one	β-elemene	
	2.2; 0.027	1.9; 0.042	1.7; 0.035	1.4; 0.025	
M. lupulina	1-octen-3-one 4.8; 0.015	β-cedrene 2.7; 0.016			
R = 0.320					
P = 0.0318					
D. glomerata	(*E*)-β-ocimene 7.2; 0.032	3-octanone 2.9; 0.022	3-carene	β-cubebene	α-muurolene 2.5; 0.008
R = 0.548			2.8; 0.025	2.6; 0.008	
P = 0.0077	(−)-α-copaene	6-methyl-5-hepten-2-one	2-pentylfuran	cadina-1(10)-4-diene	1-octen-3-ol
	2.5; 0.008	2.3; 0.008	2.2; 0.025	2.1; 0.015	2.1; 0.008
	benzaldehyde 2.1; 0.014	n.i. ionone epoxide	(*Z*)-β-ocimene 0.9; 0.017		
		1.9; 0.033			
P. angustifolia	(*Z*)-3-hexenyl acetate	limonene	hexanal	guaia-6,9-diene 3.0; 0.008	benzyl alcohol
R = 0.708	13.4; 0.016	3.4; 0.008	3.1; 0.008		2.8; 0.008
P = 0.0171	(*E*)-β-ionone	n.i. ionone epoxide	2,6-dimethyl-(3*E*,5*E*,7)-octatriene-2-ol	D-germacrene	(*Z*)-3-hexen-1-ol
	2.7; 0.008	2.6; 0.017	2.5; 0.017	2.4; 0.016	2.4; 0.027
	(±)-borneol	β-myrcene	α-pinene	2-phenyl-ethanol	β-elemene
	2.4; 0.008	2.2; 0.008	1.8; 0.015	1.8; 0.015	1.7; 0.025
	(−)-α-copaene	(*E*)-2-hexanal	sabinene	hexanoic acid	α-muurolene
	1.7; 0.016	1.7; 0.016	1.7; 0.015	1.6; 0.008	1.6; 0.026
	cadina-1(10)-4-diene	β-cubebene	elixene		
	1.2; 0.008	1.1; 0.008	0.9; 0.008		

[a] Dissimilarity of VOC profiles from WW and DT plants is indicated by ANOSIM R-values significantly different from 0.
[b] Compounds contributing significantly to blend dissimilarities based on SIMPER analyses are ranked by their estimated contribution to blend dissimilarity.

grassland species. Resistance and resilience of grassland communities to drought have been shown to be associated with the presence of mycorrhizal fungi and bacteria (Jia et al., 2020). Soil microorganisms were shown to affect above-ground volatile emissions and signalling (Babikova et al., 2014; Pineda et al., 2013, 2020; Wang et al., 2015). In our experiments, the studied plants grew in sieved, non-sterilised soil taken from the natural habitat, which simulates field conditions more closely than the use of sterilised or commercial growth media used in other studies (Kreuzwieser et al., 2021; Jin et al., 2021; Glenny et al., 2018). Thus, the microbiome typical of the plants' natural environment was allowed to colonise the roots. This may have stabilised VOC biosynthesis and emissions to a greater extent than morphological plant traits of the same set of species (Lozano et al., 2020) under drought treatment.

Second, a drought-induced reduction in aboveground plant biomass and leaf area of the studied 22 grassland species made a reduction in VOC emission from the individual plant likely. However, we based our analyses on emission rates normalised to biomass, representing a plant's relative investment into VOC and allowing for a fair comparison of different plant species. On the one hand, it is well known that drought initially can result in enhanced VOC emission rates (e.g. Blanch et al., 2009; Ebel et al., 1995) with the potential to offset the effect of reduced biomass. On the other hand, the impact of drought on VOC emissions does not result in a consistent pattern across species. Drought has also been shown to result in reduced VOC emissions (e.g., Haberstroh et al., 2018; Lavoir et al., 2009) or in no detectable effects on emissions (e.g. Peñuelas and Staudt, 2010). Thus, the overall moderate and diverse effects of drought on normalised VOC emissions are in good agreement with these divergent literature reports. Analysing the odour of any plant individual or group of plants as it is perceivable by interacting organisms would require taking absolute instead of normalised emission rates into account.

Third, acclimation to an eight-week-lasting drought period, as applied in our study, might also have contributed to the moderate effects of drought on the VOC emissions from the studied plant species. To gain deeper insight into the effects of drought on VOC emissions, it will be necessary to consider in addition to the extent of drought stress also changes in VOC emissions along a timeline from the onset of drought to its end (Niinemets, 2010). Several studies already showed that effects of drought on VOC emission can change in the course of the drought period with emission rates that increase after the onset of drought, but then decrease again when drought persists (e.g. Ormeño et al., 2007; Šimpraga et al., 2011). Hence, it is likely that the studied plant species showed different responses to the applied DT at earlier time points of the treatment.

Future studies need to elucidate how plant-associated microbes and other organisms, whose search for resources is guided by plant odorants, are affected by possible drought-induced rapid and transient changes of VOC emissions from these grassland species. In addition, it should be considered that even the moderate changes in odorant blend composition observed here after an eight-week-lasting drought period, might impact the odour landscape of the studied grassland community. The changes in VOC emissions from these species (*B. incana*, *M. lupulina*, *D. glomerata*, and *P. angustifolia*) will change ratios of compounds in the habitat odour of the grassland community.

Hence, even if many plant species would adjust their metabolism to long-lasting drought periods – as they may occur due to climate change – and do not significantly alter their VOC emissions, the moderate changes in VOC emissions of less plastic species can still affect the odour landscape of a plant community. Since host foraging behaviour of some herbivorous and also carnivorous insect species is well known to be guided by habitat odour and the ratios of its components (Schröder and Hilker, 2008), even the drought-induced change in odorant emissions of a few plant species might impact on members of the second and third trophic level. Thus, drought-induced effects on VOC emissions of only a few species within a grassland community might cause a cascade of further changes across various trophic levels.

3. Experimental

3.1. Plant species

We analysed the VOC emissions of 22 plant species frequently occurring in grassland communities in Central Europe during their vegetative phase. The analysed species belong to 12 families, of which the Poaceae were represented with seven grass species, the Asteraceae and Fabaceae each with three species, and all other families with two or one species (Table 1).

Seeds from all species were obtained from a commercial supplier specialised in producing seeds from regional sources (Rieger Hoffmann GmbH, Blaufelden-Raboldshausen, Germany), i.e. they were produced under climatic conditions like on the site outlined below. To reduce confounding effects from herbivory and other biological interactions, this study was carried out in a greenhouse setting.

3.2. Plant breeding and treatment

Sandy loam soil (% N 0.07, % C 0.77, pH 6.66) was collected in June 2016 in Dedelow, Brandenburg, Germany (53° 37′ N, 13° 77' W). All studied plant species are naturally growing at this site. The soil was sieved (4 mm mesh size) and homogenised to be used as substrate. Seeds were surface-sterilised (10% sodium hypochlorite for 5 min, 75% ethanol for 2 min, rinsing with sterile water), sown in trays with sterilised moist substrate (autoclaved at 120 °C for 20 min). Five days after germination each of ten seedlings per species was individually transplanted into a polypropylene (PP) tube (H x D: 30 cm × 11 cm) lined with polyethylene terephthalate (PET) foil. Each tube was filled with 3 L non-sterilised substrate covered with a top layer of 3 cm autoclaved (120 °C, 20 min) substrate and placed on a perforated Plexiglas disk separated by a PP tube (H: 1.5 cm) from an identical disk that was supported by another PP tube (H: 3.5 cm) standing in a pot saucer (Supplementary Fig. S1). This setup allowed the runoff of excess water.

After transplanting, each plant received 30 mL water per day for a period of four weeks. Thereafter, five individuals per species were subjected to drought treatment (DT) by gravimetrically adjusting the moisture content of the substrate in each of their respective tubes to 30% of its water holding capacity (WHC) (±0.5 g) every other day. The substrate in the tubes of the remaining five individuals per species was kept well-watered at 70% WHC (WW). These treatments lasted for another eight weeks until collection of VOC and subsequent measurement of plant traits. All tubes were randomly distributed in the greenhouse chamber. The abiotic conditions were set to: L:D 12 h:12 h (50 klx), 22 °C: 18 °C and 40% RH. All plants were in their vegetative stage, i.e., not flowering when sampling their headspace.

3.3. Plant volatile organic compound (VOC) collection

We used the following push-pull system for volatile collections. Each plant was enclosed in a PET oven bag (vol. ca. 13 L; Toppits®, Cofresco Frischhalteprodukte GmbH & Co. KG, Minden, Germany). The lower end of the bag was tightly wrapped around the top of a tube containing the plant. At the base of the bag, 300 mL/min activated charcoal-filtered air were pumped into the bags. At the upper end of the bag, 225 mL/min ±25 mL/min of VOC-laden air were drawn outside by a suction pump; thereby the air passed 5 mg activated charcoal (Brechbühler AG, Schlieren, Switzerland), where VOC were trapped. PTFE tubing was used to connect the different parts of the setup, and care was taken to tightly seal all junctions. After enclosing individual plants in the odorant collection system (Supplementary Fig. S1), they were given 8 h to acclimatise before odorant collections started at 4 a.m. for 16 h encompassing the entire light period, which started at 6 a.m. and lasted for 12 h plus 2 h of the scotophase before and after the photophase.

Trapped volatile compounds were extracted from the activated charcoal filters with 25 μL dichloromethane containing 1 ng/μL of the internal standard (IS) methyl nonanoate (98%, Sigma-Aldrich, München, Germany). The samples were stored at −80 °C until analysis.

From each plant species, VOC were sampled from $N = 5$ individuals exposed to the drought treatment and $N = 5$ individuals that were well watered.

3.4. Identification of VOC

VOC analyses were performed by GC-MS. We injected an aliquot of 1 μL per sample in splitless mode into the inlet (250 °C) of a GC (Agilent 7890A) coupled to a MS (Agilent 5975C triple axis detector). Separation was achieved on a Zebron ZB-5HT column (30 m, 250 μm ID, 0.25 μm active phase) under constant flow (1 mL/min) with a programmed temperature ramp (40 °C, 4 min, 5 °C/min to 300 °C maintained for 10 min). The transfer line to the MS was kept at 300 °C. Total ion current chromatograms (TICs) were recorded after a solvent delay (4 min) in EI mode (70 eV, electron source at 230 °C, quadrupole at 150 °C) covering 35 to 450 AMU. Mixtures of unbranched alkanes (10 ng/μL each of C7–C40) were injected at regular intervals to calculate linear retention time indices (RI) for all peaks (Van den Dool and Kratz, 1963).

VOC were identified or tentatively identified by comparing their mass spectra and RI to authentic reference substances or library records (NIST Mass Spectral Search Program Vers. 2.2; NIST library 2014), respectively. Peaks of interest that could not be identified following this procedure were assigned to compound classes based on characteristic *m/z* peaks in their mass spectra and tagged with a unique identifier for statistical analyses. Co-eluting compounds were systematically detected using the AMDIS deconvolution software (Vers. 2.70, 2011). Based on the chemical structure (e.g. silanes from column bleeding or septum) or based on their known non-organismic origin (e.g. 2-ethylhexan-1-ol, phthalates, etc. (Horn et al., 2004; Nalli et al., 2006), compounds were considered as contaminants and excluded from further analyses.

3.5. Semiquantitative VOC analysis

In a first step, peak areas in the TICs were integrated using Agilent's ChemStation Software (version F.February 01, 2357; settings: initial area reject 100,000; initial peak width 0.004; shoulder detection off; initial threshold 13.7). Thereafter, the area of all peaks of VOC were compared to the area of the IS of the same sample. If the ratio was at least 10:1 in any sample, the respective compound was identified and semi-quantified in all samples down to the detection limit. Furthermore, compounds had to be detected in at least two samples of a species to be considered part of the VOC emissions of the species and to be included in further statistical analyses.

If a peak consisted of co-eluting compounds, their relative contribution to the TIC peak was estimated as follows: identification of the base peak in the mass spectrum of each co-eluent; integration of the corresponding single ion *m/z*-traces; partitioning of the TIC peak area according to the ratio of base peak areas of the co-eluents.

To semi-quantify the VOC per sample, a calibration curve was calculated from peak areas resulting from 0.1, 1, 10, 50, 100 and 500 ng of the IS per 1 μL injection volume ($N = 3$ runs per dilution step). All instrument settings were identical to those described above. This calibration curve enabled semi-quantifying a compound of interest present in a sample as internal standard equivalents.

Emission rates of VOC were correspondingly calculated as internal standard equivalents normalised to dry biomass of the sampled plant and sampling duration (16 h). These values served as input to all graphics and statistical evaluations.

3.6. Richness and chemical diversity

The chemical richness of a sample was determined as the number (n) of different VOC present in that sample and fulfilling the quantitative criterion for including compounds in the analyses (see 3.5).

The chemical diversity of a sample was calculated as Shannon diversity index as follows:

$$H' = -\sum_{n=1}^{n} p_i * ln\ p_i$$

H' is the diversity index, n is the number of compounds semi-quantified in a sample, p_i is the proportion by which each compound contributes to the total emission rate of that sample, and $\ln p_i$ is the natural logarithm of this proportion. This index takes compound richness and evenness of odorant profiles into account.

Additionally, richness and chemical diversity were determined separately for each main biosynthesis pathway to test for phylogenetic signals at this subordinate level (see 3.9).

3.7. Total emission rates and emission rates by compound classes

In addition to the total VOC emission rate per plant and collection time (16 h), we determined emission rates of individual VOC and of the sum of VOC belonging to the same compound class.

Total emission rates (mean ± SE) were calculated as the sum of all semi-quantified compounds present in each sample and averaged for each species ($N = 5$ DT plants, 5 WW plants). Correspondingly, emission rates per compound class were calculated as sum of the emission rates of all semi-quantified compounds belonging to that class. Assignment to compound classes was based on the biosynthetic origin (pathway) as reviewed by Junker et al. (2017). Fatty acid derivatives (FAD) including green leaf volatiles (GLV) were considered products of the lipoxygenase (LOX) pathway (Matsui, 2006). Mono- and diterpenes as well as the detected volatile derivatives of tetraterpenes (carotenoid derivatives) were classified as products of the 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway (Dudareva et al., 2005). Sesquiterpenes and their derivatives including the triterpene squalene were products of the mevalonate (MEV) pathway (Dudareva et al., 2005). Aromatic volatiles are produced via the shikimate (SHI) pathway (Maeda and Dudareva, 2012; Vogt, 2010). Aliphatic hydrocarbons (HC) (excluding terpenes and functionalized HC like GLV) may arise from various precursors and are therefore listed as a separate compound class. Other compounds, which could not reliably be assigned to any of the above-mentioned pathways, were classified as diverse (Div) (Charpentier et al., 2012).

3.8. Plant volatile profiles with respect to their biosynthetic pathway and the plant's phylogeny

We referred to the VOC's biosynthetic pathways to analyse (i) how VOC sorted by their biosynthetic pathway differ across plant species (DT plants, WT plants), (ii) how the emission of these biosynthetically different VOC is affected by drought, (iii) whether drought-induced changes in emission of biosynthetically different VOC reflect plant phylogeny.

We applied a biosynthetically informed distance measure recently developed and validated by Junker (2018). In brief, this concept treats compounds sharing the same biosynthetic pathway as more similar to each other than compounds from different pathways. Correspondingly, two samples may yield a high similarity value if all their different compounds emanate from the same pathway and share a wide array of biosynthetic enzymes. The concept has been extended to datasets with known main pathways but unknown enzymatic pathway details (functions ‚biosyntdist' and ‚mergedist'; R-package GUniFrac Chen, 2012; Junker, 2018).

For WW and DT plants, dendrograms were constructed based on clustered biosynthetically informed sample similarities (function ‚hclust'; method ‚average'; R-package vegan, Oksanen, 2016) with 'species' set as grouping variable. To assess whether VOC blend compositions were shaped by the plant's phylogeny, these dendrograms

were compared to the phylogenetic tree of the plants under investigation (see 3.9) by generating tanglegrams (function ‚untangle', R-package dentextend Galili, 2015). Beyond VOC emission rates under each treatment (WW and DT) drought-induced changes may be under phylogenetic control. Therefore, we also constructed a dendrogram based on the differences of emission rates between plants under both treatments and compared it to the phylogenetic tree described above.

Furthermore, we performed an analysis of similarity of entire VOC profiles of DT and WW plants within species using the function ‚anosim' (R-package vegan) which is based on the Bray-Curtis distance measure. We tested the null hypothesis that average ranks of similarities of VOC blends emitted by individuals from different treatment groups are greater or equal compared to similarities of VOC blends emitted by individuals from the same treatment group. The Bray-Curtis distance represents a valuable measure to quantify (dis)similarities of blends of VOC irrespective of their biosynthetic origin, and is defined as

$$d_{ij} = \frac{\sum_{k=1}^{p} |y_{ik} - y_{jk}|}{\sum_{k=1}^{p} (y_{ik} + y_{jk})},$$

where d_{ij} corresponds to the distance between sample i und j, p corresponds to the total number of compounds present in both samples, and k is an enumerator for each compound. For compounds significantly contributing to dissimilarities of the VOC profiles ‚similarity percentages' (function ‚simper', R-package vegan) were calculated (Clarke, 1993).

3.9. Phylogenetic analysis

A phylogenetic tree for the species under investigation (Table 1) was extracted from 'DaPhnE', a phylogeny of the central European flora. DaPhnE has been constructed by consolidating several hundred molecular studies into one dated ultrametric phylogenetic tree and was conceived for phylogenetically informed comparative analyses of trait correlations in 'R' (Angiosperm Phylogeny Group, 2009; Durka and Michalski, 2012). The resulting tree was used to create tanglegrams comparing phylogenetic relationships to VOC profiles (Fig. 3, Supplementary Fig. S3).

While these tanglegrams elucidate whether similar VOC profiles match close plant phylogenetic relationships of the investigated species, emissions from specific VOC classes and chemical diversity within VOC classes may be under phylogenetic control without translating into similarity of an entire VOC profile.

To test for phylogenetic signals in VOC blends, we used the ‚phylosig' function (R-package phylosignal; Keck et al., 2016). It calculates Pagel's λ, which indicates to what extent the covariation of a trait is under phylogenetic control. Pagel's λ corresponds to a scaling factor fitting branch lengths of a given tree to a tree obtained under a Brownian motion model (Pagel, 1999). We searched for phylogenetic signals in the patterns of VOC emissions of WW and DT plants with respect to (i) richness, (ii) Shannon diversity, and (iii) summarised emission rates within compound classes (Supplementary Table S2).

3.10. Drought-induced changes in VOC emissions and their relationship to plant traits

Drought induced changes in emission of VOC and other plant traits were normalised by applying the relative interaction intensity index RII as described by Armas et al. (2004):

$$RII = \frac{(Y_D - Y_W)}{(Y_D + Y_W)},$$

where Y_D and Y_W correspond to quantitative trait values measured under DT and under WW conditions, respectively. RII values around zero indicate no change, while values approaching −1 or +1 indicate a strong negative or positive impact of drought on the measured trait. Unless otherwise stated, all trait changes of a species are expressed as RII mean calculated from RII values for each of all possible pairs of plants individuals (permutations) submitted to WW or DT conditions.

We related VOC emission rates to the following plant traits: aboveground plant biomass, specific leaf area (SLA), and leaf dry matter content (LDMC) as measured by Lozano et al. (2020), who used the same plant individuals and the same experimental setup as we did. In our study, we additionally determined the total water consumption of each plant under DT and WW treatment as sum of the water volume required every other day to moisten the substrate to 30% WHC (DT) and 70% WHC (WW), respectively. To determine whether the abovementioned plant traits are associated with distinct VOC patterns, plants were clustered by their traits under each condition, and the resulting dendrograms were compared to the dendrogram based on biosynthetically informed distances in tanglegrams (function ‚untangle', R-package dentextend Galili, 2015).

3.11. Statistics

All statistics and graphs have been performed or created using 'R' statistical software (R Core Team, 2021; Version 4.1.2). Compounds concentrations had non-homogenous variances (Levene's test) but were normally distributed (Shapiro-Wilk test). One sample *t*-test was applied to assess whether RII values for traits under investigation were significantly different from zero (indicating a neutral or non-significant effect).

Colour coded figures were created using the R-package pheatmap with the function 'pheatmap', with the sequence of rows corresponding to the plant species' phylogenetic tree (Fig. 3) and columns representing the variables or compounds under consideration.

CRediT authorship contribution statement

Andreas Reinecke: Writing – review & editing, Writing – original draft, Validation, Formal analysis, Data curation. **Isabelle C. Flaig:** Writing – review & editing, Visualization, Investigation, Formal analysis. **Yudi M. Lozano:** Writing – review & editing, Investigation. **Matthias C. Rillig:** Writing – review & editing, Project administration, Funding acquisition, Conceptualization. **Monika Hilker:** Writing – review & editing, Supervision, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

This study was funded by the German Federal Ministry of Education and Research (BMBF) within the Collaborative Project "Bridging in Biodiversity Science - BIBS" (funding number 01LC1501). We thank two anonymous referees for their highly appreciated constructive review of our manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.phytochem.2024.114040.

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