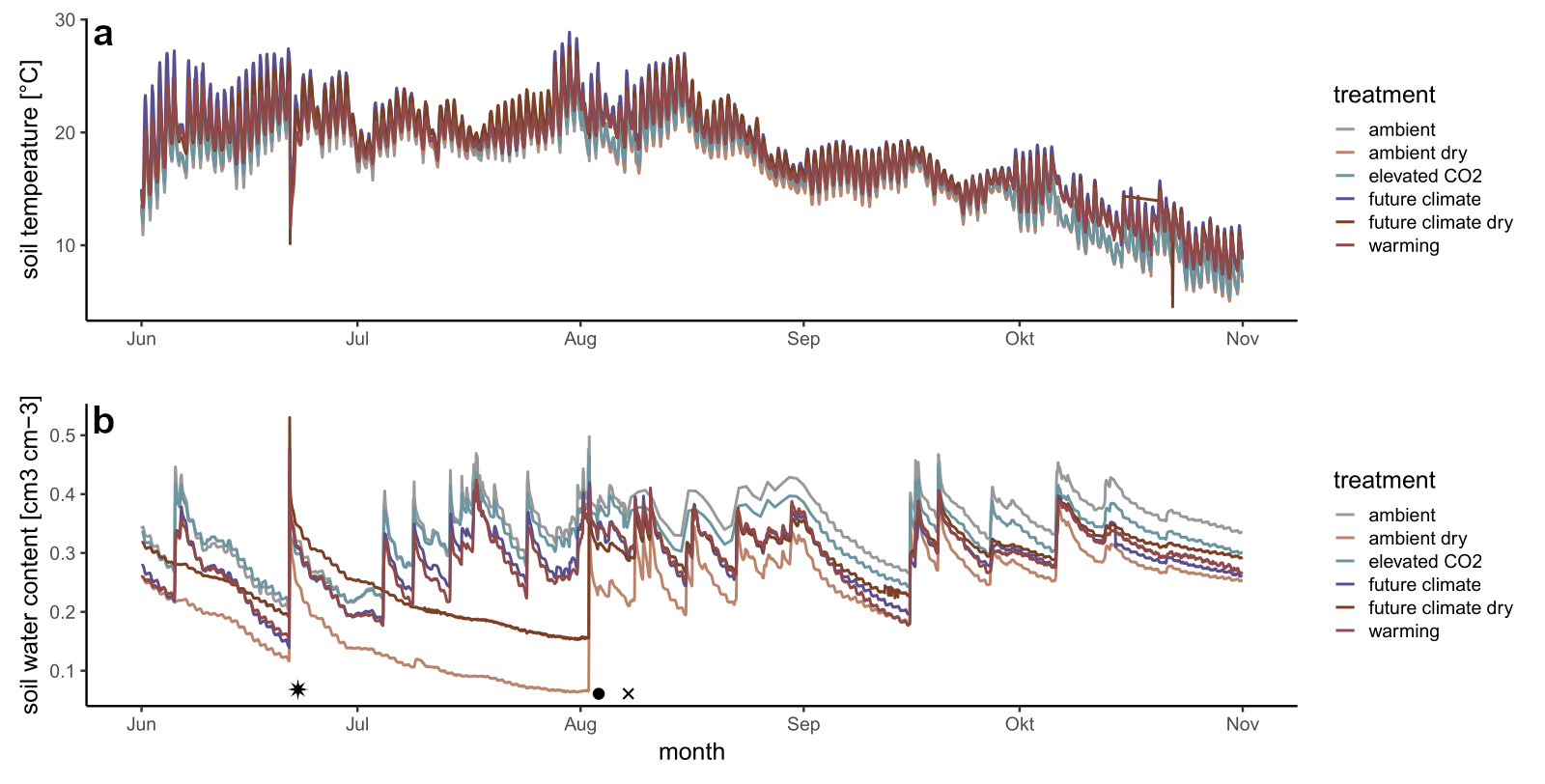
# **Table S1. Reagents and Standards**

|  |  |
| --- | --- |
| **Chemicals** | **Vendor** |
| Water (LC-MS) | Honeywell ( North Carolina, USA) |
| Isopropanol (LC-MS) | Honeywell ( North Carolina, USA) |
| Acetonitrile (LC-MS) | Merck Millipore (Darmstadt, Germany) |
| Formic acid | Honeywell ( North Carolina, USA) |
| Methanol | Sigma Aldrich (St. Louis, USA) |
| Chloroform | Sigma Aldrich (St. Louis, USA) |
| Citric acid monohydrate | Sigma Aldrich (St. Louis, USA) |
| Ammonium formate | Sigma Aldrich (St. Louis, USA) |
| Sodium Hydroxide | Sigma Aldrich (St. Louis, USA) |

|  |  |
| --- | --- |
| **Lipid Standards** | **Vendor** |
| 15:0-18:1(d7) PC | Avanti Polar Lipids (Birmingham, USA) |
| 18:1(d7) Lyso PC | Avanti Polar Lipids (Birmingham, USA) |
| 15:0-18:1(d7) PE | Avanti Polar Lipids (Birmingham, USA) |
| 18:1(d7) Lyso PE | Avanti Polar Lipids (Birmingham, USA) |
| 15:0-18:1(d7) PG (Na Salt) | Avanti Polar Lipids (Birmingham, USA) |
| 15:0-18:1(d7) PI (NH4 Salt) | Avanti Polar Lipids (Birmingham, USA) |
| 15:0-18:1(d7) PS (Na Salt) | Avanti Polar Lipids (Birmingham, USA) |
| 15:0-18:1(d7)-15:0 TAG | Avanti Polar Lipids (Birmingham, USA) |
| 15:0-18:1(d7) DAG | Avanti Polar Lipids (Birmingham, USA) |
| 18:1(d7) MAG | Avanti Polar Lipids (Birmingham, USA) |
| 18:1(d7) Chol Ester | Avanti Polar Lipids (Birmingham, USA) |
| d18:1-18:1(d9) SM | Avanti Polar Lipids (Birmingham, USA) |
| C15 Ceramide-d7 | Avanti Polar Lipids (Birmingham, USA) |
| 15:0-18:1 PC | Avanti Polar Lipids (Birmingham, USA) |
| 18:1 Lyso PC | Avanti Polar Lipids (Birmingham, USA) |
| 15:0-18:1 PE | Avanti Polar Lipids (Birmingham, USA) |
| 18:1 Lyso PE | Avanti Polar Lipids (Birmingham, USA) |
| 15:0-18:1 PG | Avanti Polar Lipids (Birmingham, USA) |
| 15:0-18:1 PI | Avanti Polar Lipids (Birmingham, USA) |
| 15:0-18:1 PS | Avanti Polar Lipids (Birmingham, USA) |
| 15:0-18:1-15:0 TG | Avanti Polar Lipids (Birmingham, USA) |
| 15:0-18:1 DG | Avanti Polar Lipids (Birmingham, USA) |
| 18:1 MG | Avanti Polar Lipids (Birmingham, USA) |
| 18:1 Chol Ester | Avanti Polar Lipids (Birmingham, USA) |
| d18:1-18:1 SM | Avanti Polar Lipids (Birmingham, USA) |
| C15 Ceramide (d18:1/15:0) | Avanti Polar Lipids (Birmingham, USA) |
| Sphingosine (C17 base) | Avanti Polar Lipids (Birmingham, USA) |
| Sphinganine (C17 base) | Avanti Polar Lipids (Birmingham, USA) |
| Sphingosine-1-P (C17 base) | Avanti Polar Lipids (Birmingham, USA) |
| Sphinganine-1-P (C17 base) | Avanti Polar Lipids (Birmingham, USA) |
| Lactosyl(ß) C12 Ceramide | Avanti Polar Lipids (Birmingham, USA) |
| 12:0 Sphingomyelin | Avanti Polar Lipids (Birmingham, USA) |
| Glucosyl(ß) C12 Ceramide | Avanti Polar Lipids (Birmingham, USA) |
| 12:0 Ceramide | Avanti Polar Lipids (Birmingham, USA) |
| 12:0 Ceramide-1-P | Avanti Polar Lipids (Birmingham, USA) |
| 25:0 Ceramide | Avanti Polar Lipids (Birmingham, USA) |
| Monogalactosyldiacylglycerol (Plant), powder | Avanti Polar Lipids (Birmingham, USA) |
| Digalactosyldiacylglycerol (Plant), powder | Avanti Polar Lipids (Birmingham, USA) |
| Sulfoquinovosyldiacylglycerol, powder | Avanti Polar Lipids (Birmingham, USA) |
| 1,2-dipalmitoyl-*sn*-glycero-3-O-4′-(N,N,N-trimethyl)-homoserine | Avanti Polar Lipids (Birmingham, USA) |
| Sphingosine (C17 base) | Avanti Polar Lipids (Birmingham, USA) |
| Sphinganine (C17 base) | Avanti Polar Lipids (Birmingham, USA) |
| Sphingosine-1-P (C17 base) | Avanti Polar Lipids (Birmingham, USA) |
| Sphinganine-1-P (C17 base) | Avanti Polar Lipids (Birmingham, USA) |
| Lactosyl(ß) C12 Ceramide | Avanti Polar Lipids (Birmingham, USA) |
| 12:0 Sphingomyelin | Avanti Polar Lipids (Birmingham, USA) |
| Glucosyl(ß) C12 Ceramide | Avanti Polar Lipids (Birmingham, USA) |
| 12:0 Ceramide | Avanti Polar Lipids (Birmingham, USA) |
| 12:0 Ceramide-1-P | Avanti Polar Lipids (Birmingham, USA) |
| 25:0 Ceramide | Avanti Polar Lipids (Birmingham, USA) |
| 17:1 Lyso PG (Na salt) | Avanti Polar Lipids (Birmingham, USA) |
| 17:1 Lyso PA (NH4 salt) | Avanti Polar Lipids (Birmingham, USA) |
| 17:1 Lyso PI (NH4 salt) | Avanti Polar Lipids (Birmingham, USA) |
| 17:1 Lyso PS (Na salt) | Avanti Polar Lipids (Birmingham, USA) |
| 17:1 Lyso PC | Avanti Polar Lipids (Birmingham, USA) |
| 17:1 Lyso PE | Avanti Polar Lipids (Birmingham, USA) |
| 17:0-17:0 DAG | Avanti Polar Lipids (Birmingham, USA) |
| 17:0-17:0-17:0 TAG | Avanti Polar Lipids (Birmingham, USA) |
| 12:0 SM (d18:1/12:0) | Avanti Polar Lipids (Birmingham, USA) |
| 17:0-14:1 PC | Avanti Polar Lipids (Birmingham, USA) |
| 17:0-14:1 PS (NH4 salt) | Avanti Polar Lipids (Birmingham, USA) |
| 17:0-14:1 PG (NH4 salt) | Avanti Polar Lipids (Birmingham, USA) |
| 17:0-14:1 PA (NH4 salt) | Avanti Polar Lipids (Birmingham, USA) |
| 17:0-14:1 PE | Avanti Polar Lipids (Birmingham, USA) |
| 17:0-14:1 PI (NH4 salt) 250 | Avanti Polar Lipids (Birmingham, USA) |
| 17:0 cholesterol ester | Avanti Polar Lipids (Birmingham, USA) |
| NIST® SRM® 1950 | Sigma Aldrich (St. Louis, USA) |

Figure S1

Microclimate

****

Soil moisture and temperature were constantly (hourly) monitored at the ‘ClimGrass’ field site. Here, microclimate data between June 1st 2021 (before the rainout shelters were set up) and October 31st, 2021 are shown. Soil temperature (average of soil depths 3 and 9 cm) was higher (109.1%) in all warmed plots (warmed, future and future-drought) over the course of the sampling period (August 2nd 2021 to August 6th 2021, Fig. 1a).

Plots without drought treatment showed overall higher soil moisture content over the course of the experimental period than drought treated plots. Fluctuations were caused by precipitation events. Ambient and eCO2 plots showed similar soil moisture dynamics, while plots with increased temperature (warming and future climate) showed, relative to ambient conditions, decreased soil moisture (62.6%). The drought treated plots (both ambient and future climate) had lower soil moisture values almost immediately after the setup of the rainout shelters (marked with an ✷ in the graph), drought and future climate in combination reduced the decrease in soil moisture. The steep increase in soil moisture in early August marks the rewetting experiment (Fig. 1b).

Table:1 Sample used for lipid extraction

|  |  |  |  |
| --- | --- | --- | --- |
| Sample | Type1 | Type2 | Weight for lipid extraction (g) |
| 19 | Ambient | No\_drought | 2.054 |
| 22 | Ambient | No\_drought | 1.965 |
| 36 | Ambient | No\_drought | 2.063 |
| 28 | Ambient | Drought | 2.048 |
| 44 | Ambient | Drought | 2.063 |
| 49 | Ambient | Drought | 2.082 |
| 35 | Future | No\_drought | 2.065 |
| 40 | Future | No\_drought | 2.032 |
| 52 | Future | No\_drought | 2.033 |
| 27 | Future | Drought | 2.071 |
| 43 | Future | Drought | 2.028 |
| 48 | Future | Drought | 2.027 |
| 50 | Future | Drought | 0.178 |

**Table S2. UHPLC gradients.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Mobile Gradient - LC-orbitrap** | | | |
| Time (min) | Mobile phase %B | Flow (ul/min) | To |
| 0 | 30 | 300 | Waste |
| 1 | 30 | 300 | MS |
| 18 | 85 | 300 | MS |
| 20 | 90 | 300 | MS |
| 24 | 90 | 300 | MS |
| 28 | 30 | 300 | WASTE |
| 30 | 30 | 300 | END |
|  |  |  |  |
| Sample injection | 5 ul |  |  |
| column oven temp (in Celsius) | 40 |  |  |
| Auto sampler temp (in Celsius) | 10 |  |  |

|  |  |  |
| --- | --- | --- |
| **Table S3.** | **MS Parameters for Q Exactive** |  |
|  | Top 5 |  |
| Polarity | Positive | Negative |
|  | Full Scan | Full Scan |
| Resolution | 70000 | 70000 |
| AGC | 1.00E+06 | 1.00E+06 |
| Maximum IT(ms) | 50 | 50 |
| Scan range (m/z) | 300-1800 | 300-1800 |
|  | TopN (ddMS2) | TopN (ddMS2) |
|  | 5 | 5 |
| Resolution | 17500 | 17500 |
| AGC | 1.00E+05 | 1.00E+05 |
| Maximum IT(ms) | 117 | 117 |
| Isolation window (m/z) | 1 | 1 |
| NCE | 25,30 | 25,30 |
| Dynamic exclusion (s) | 20 | 20 |
| Minimum AGC | 2.00E+02 | 2.00E+02 |
|  |  |  |
| AGC | Automatic gain control |  |
| IT | Injection time |  |
| NCE | Normalized collision energy |  |

|  |  |
| --- | --- |
| **Table 2 Lipid Nomenclature of different lipid classes in this study** | |
| Lipid Class | Abbreviation |
| Lysophosphatidylcholine | LPC |
| Lysophosphatidylethanolamine | LPE |
| Lysophosphatidic Acid | LPA |
| Lysophosphatidylglycerol | LPG |
| Lysophosphatidylinositol | LPI |
| Lysophosphatidylserine | LPS |
| Phosphatidylcholine | PC |
| Phosphatidylethanolamine | PE |
| Phosphatidylglycerol | PG |
| Phosphatidylinositol | PI |
| Phosphatidylserine | PS |
| Phosphatidic Acid | PA |
| Ceramide | CER |
| Sphingosine | SPR |
| Sphingosine | SPH |
| Ceramide Phosphate | CerP |
| Glucosylceramide | GlcCer |
| Lactosyl-ceramide | LacCer |
| Sphingomyelin | SM |
| Digalactosyldiacylglycerol | DGDG |
| Sulfoquinovosyldiacylglycerol | SQDG |
| Diacylglyceryl-N,N,N-trimethylhomoserine | DGTS |
| Monogalactosyldiacylglycerol | MGDG |
| Monoacylglycerol | MG |
| Triglyceride | TG |
| Diacylglycerol | DG |
| Cholesteryl Ester | CE |
| Fatty acyl chain | FA |
| Steryl ester | SE |
| Phospholipid | PL |
| Cardiolipin | CL |
| Sphinganine-1-Phosphate / Sphingosine-1-Phosphate | LCBP |
| Long-chain base (sphingoid bases) | LCB |
| Mannosyl-Diinositolphosphoceramide | M(IP)2C |
| Mannosyl-Inositolphosphoceramide | MIPC |
| Inositolphosphoceramide | IPC |

|  |  |
| --- | --- |
| **ClassKey** | **notation** |
| AcHexCmE | AcHexCmE |
| AcHexSiE | AcHexSiE |
| CDP-glycerols | CDP-glycerols |
| Cucurbitacins | CE |
| Cer | Cer |
| Ceramides | Cer |
| CerP | CerP |
| Cholestane steroids | Cholestane |
| CL | CL |
| Co | Co |
| DG | DG |
| Diradylglycerols | DG |
| Glycosylglycerols | DG |
| DGDG | DGDG |
| Glycerol ethers | DG-dO |
| FA | FA |
| Fatty acids and conjugates | FA01-A |
| Lineolic acids and derivatives | FA01-B |
| Eicosanoids | FA03 |
| Fatty alcohols | FA05 |
| Fatty acid esters | FA07-A |
| Fatty acyl thioesters | FA07-B |
| Fatty alcohol esters | FA07-C |
| Fatty amides | FA08 |
| Fatty acyl glycosides | FA13 |
| Hex1Cer | Hex1Cer |
| Hex2Cer | Hex2Cer |
| Glycosphingolipids | HexCer |
| LPA | LPA |
| LPC | LPC |
| LPE | LPE |
| LPG | LPG |
| LPI | LPI |
| MG | MG |
| Monoradylglycerols | MG |
| Glycerophosphates | PA |
| PA | PA |
| Glycerophosphocholines | PC |
| PC | PC |
| Glycerophosphoethanolamines | PE |
| PE | PE |
| Glycerophosphoglycerols | PG |
| PG | PG |
| Glycerophosphoinositols | PI |
| PI | PI |
| Monoterpenoids | PR0102 |
| Sesquiterpenoids | PR0103 |
| Diterpenoids | PR0104 |
| Sesterterpenoids | PR0105 |
| Triterpenoids | PR0106 |
| Tetraterpenoids | PR0107 |
| Polyterpenoids | PR0108 |
| Polyprenols | PR01-A |
| Terpene glycosides | PR01-B |
| Quinone and hydroquinone lipids | PR02 |
| Glycerophosphoserines | PS |
| PS | PS |
| Phosphosphingolipids | SM |
| SM | SM |
| SPH | SPH |
| SQDG | SQDG |
| Ecdysteroids | ST0101 |
| Ergostane steroids | ST0103 |
| Stigmastanes and derivatives | ST0104 |
| Spirostanes and derivatives | ST0108 |
| Oxosteroids | ST01-A |
| Hydroxysteroids | ST0203 |
| Pregnane steroids | ST0203 |
| Steroid esters | ST02-A |
| Steroid lactones | ST02-B |
| Steroidal alkaloids | ST02-C |
| Steroidal glycosides | ST02-D |
| Delta-5-steroids | ST02-E |
| Vitamin D and derivatives | ST03 |
| Steroid acids | ST04 |
| Sulfated steroids | ST0502 |
| Androstane steroids | ST-C19 |
| C24-propyl sterols and derivatives | ST-C24 |
| Bile acids, alcohols and derivatives | ST-C26 |
| TG | TG |
| Triradylcglycerols | TG |