9 Micro-controllers and Sensors for Novel Crop Quality Assessments: Volatiles and CO_2

9.1 Introduction

Integrated networks and hardware can allow for effective control of critical glass house components. These systems, while common in many commercial glass houses, are limited. Large capital costs, complicated system maintenance and specialist contractors can have the potential to stagger crop cycles if failure occurs.

Horticultural management systems (HMS), also known as nursery management systems (NMS) are integrated control platforms that allow access and manipulation of various parameters of the growing environment. This may include lighting, humidity, shade and temperature. Often these systems will center around irrigation management, whether this be control of flood benches, booms or misting.

This study takes HMS/NMS further, by addressing the potential of real-time management and assessment of croppings through unique observational data, in this instance; Volatile and CO_2, and light data.

9.2 Potential of Sensor Technology in Crop Production

Development of sensors and new technologies for successful crop production has expanded over recent years. These interest have been pushed forward by the acceleration and cost reduction of Drone or Unmanned Aerial Vehicle (UAV) technology, and its use in agriculture (Veroustraete 2015). Adding camera's (such as infrared), LIDAR (light detection and ranging) and GIS (geographical information systems), UAV's are now able to 3D map fields, produce heat maps for crop quality, direct precision pesticide and fertilizer use, as well as produce maps on soil quality (Puri, Nayyar, and Raja 2017). This potential in crop assessment, yield increase and cost savings measures has been somewhat stifled by the limitation and licensing of UAV use for commercial and domestic entities over security, safety and privacy concerns (West et al. 2019; Hoenig 2014; Schlag 2013).

Several sensing technologies are already used in horticultural practices, often in NMS's, the presence of temperature, humidity and light intensity sensors are common place. These sensors inform other parts of the NMS to either enhance shade cover when sunlight may damage crops, increase heating if the facility is too cold or increase misting if humidity is too low (Verdouw, Robbemond, and Kruize 2015). These systems, although common and extremely useful in successful nursery management, are missing out on a quiet revolution for easily interfaced, small form-factor controllers and the interfacing with cost-effective, precise sensoring technologies (Brown 2012).

This element of precision application and assessment, using various technologies has been translated to the horticulture sector, with interest in sensor technology for real time sensoring of novel and unique crop quality information; "the monitoring of multicomponent chemical media" (Snopok and Kruglenko 2002). In essence, these sensors are often either targeted for specific components in gas, or background/'cloud' sensing. Wherein quantitative data of overall Volatiles are observed, rather than in targeted systems found in industrial facilities.

In horticulture, often analytically tool technology is used invasively, outside of the growing environment to gather data, often destroying the crop in the process (Ruiz-Altisent et al. 2010). Nuclear magnetic resonance (NMR) and Gas chromatography–mass spectrometry (GC/MS) for example.

The principle of using gas sensors such as the SGP30 is to demonstrate the ability for early plant stress detection (Catini et al. 2019), such as drought, pest activity or pathogens (Khater, Escosura-Muñiz, and Merkoçi 2017). This information may also be used to observe treatment effects in real-time, effectively allowing for treatment development to be on-going, during the trial period. The reasoning for using this technology and approach was developed out of the COVID-19 pandemic, and a sudden loss of access to commercial facilities for planned trials. This approach would allow the study to continue in a different, yet novel direction. Allowing for testing an approach that may give information regarding treatment effect when there is lacking visual evidence (such as demonstrated through the PGPR's in Basil).

9.3 Method and Materials

The premise of using technologies such as the microcontrollers/sensors evolved out of the nessicity for finer treatment effect data. The Covid-19 outbreak resulted in the loss of access to commercial growth facilities, upsetting further full-scale trial developments. As a response to this, development of sensor technology evolved into a tangible answer to observing treatment effects in real-time, on a non-visual level.

Two growing media were used for this trial. A commercial Peat substrate sourced from a significant producer of potted plants, and a hand-blended mix of organic, mineral and fertilizer products to create a Peat-Free growing media. This Peat-Free growing media was mixed to replicate a domestically available product, sold by Bulrush LTD. This product aims to mitigate the negative impacts on global climate created by the use of Peat based growing media, the production of which can be attributed to significant green house gas emissions.

Sensor and control components

The bases of this trial is a single-chip micro-controller: ATmega328. This chip is used in this study as part of the Arduino family series of bread-board friendly, GPIO interfacing platforms, specifically the Arduino Nano.

The structure for this system relied heavily on the SGP30 air quality sensor developed by Sensirion. The SGP30 is a micro leadframe (MLF), otherwise known as a Dual flat no lead (DFN); a type of flat integrated circuit used for mounting on PCB boards for use in electronics. The exceptionally small form factor of most MLF's mean they are perfect for use in sensoring technology and mounting on circuit integrators such as the I^2C for communication to micro-controllers such as the Arduino family. The operation of an SGP30 is through the use of a micro-hotplate to volatilize the samples for processing via air quality signals.

The difficulty in using multipul I^2C devices of the same type is the limitation posed by the address system developed for them. Each new device is designated an address, some fixed, others not. When a device has a fixed address, the SDA (serial data line) and SLC (serial clock line) cannot be shared by multiple devices with the same address. In the interests of costs and power management, use of multiple micro-controllers for individual SGP30's was not practical. The solution was to implement the use of a multiplexer (in this case a TCA9548A clone) to allow for multiple SDA/SLC connections to be selected and forwarded along a single output (I.E. arduino SLC/SDA analogue pins; A4/A5, respectively)

Organisms

Arbusucular Mycorrhizal Fungi (AMF) were used as a biological treatment for this study. A granular medium was used as an inoculum for introducing AMF spores into both Peat and Peat-Free growing medias. This was applied as per the manufucateres guidelines, added as a layer beneath the sowing level for crop seeds.

Both Basil and Coriander croppings were assessed for real-time Volatile and CO_2 levels. Only Basil is shown here due to significant water damage to digital media components for Coriander croppings.

Qualatative GC-MS

A polymer coated filament was employed for solid phase microextraction (SPME), a Hewlett Packard 5890 GC-MS system was used with in conjunction with the filament absorbency tool to absorb volatiles produced from C. sativum. This was achieved by harvesting shoots and leaves, weighing to maintain an equal sample size (3g .01) and placing in an inert ‘oven bake’ plastic bag and leaving for 20minutes (see Figure 69). After this time, volatiles in the bag would have accumulated allowing for collection via the filament. The running program for analysis was 50C initial temperature (held for 2mins) with a final temperature of 280C, rising at 10C minute.

Figure 69: SPME probe absorbing gas emissions from inert plastic bag with harvested Coriander.

9.4 Design

The trials for gas levels were set up for *ca.* 45 days of growth, to imitate the approxiamte length of the lifetime of a potted herb plant from initial growth to supermarket. The trials were not randomised (see Figure 70) due to the necessity to have each treatment within a specificy proximity to a sensor.

(a) (b)

Figure 70: None randomised layout for Gas sensor trials. Pots of the same treatment are oreintated around each gas sensor in blocks. Gas sensor can be seen at approximate crop height on upturned pot.

9.5 Interfacing

Integrated development environments (IDEs) are critical software that provide interfacing environments to work the micro-controller elements for sensor's. Typically consisting of a terminal window, editor and code debugging highlight features, several were used for the development of this system.

The Ardunio IDE (C/C++) was used for control of the Nano clone and Python 3.7 was selected for its simple shell function and interfacing ability with Raspbian OS terminal functionality, a critical component for remote secure shell (SSH) connection.

The Nano, once programmed through the Arduino IDE was selected to communicate directly through a serial interface (USB) rather than GPIO options such as I^2C channels (SDA/SLC). This was to ensure a robust physical system, as well as to ensure easily monitored live sensor information through the terminal via SSH.

The SSH was formed using PuTTY between a remote desktop environment (Windows 10) and the Raspberry Pi. This system was abandoned after increasing issues between the host (Pi) and remote access due to interference caused by the glasshouse facilities localized VPN.

9.6 Set-Up

Peat and peat-free substrates were used in tamdem with Arbuscular Mycorrihzal Fungi (AMF), in 0.5L pots with basil seeds for Tvoc/CO2 analysis.

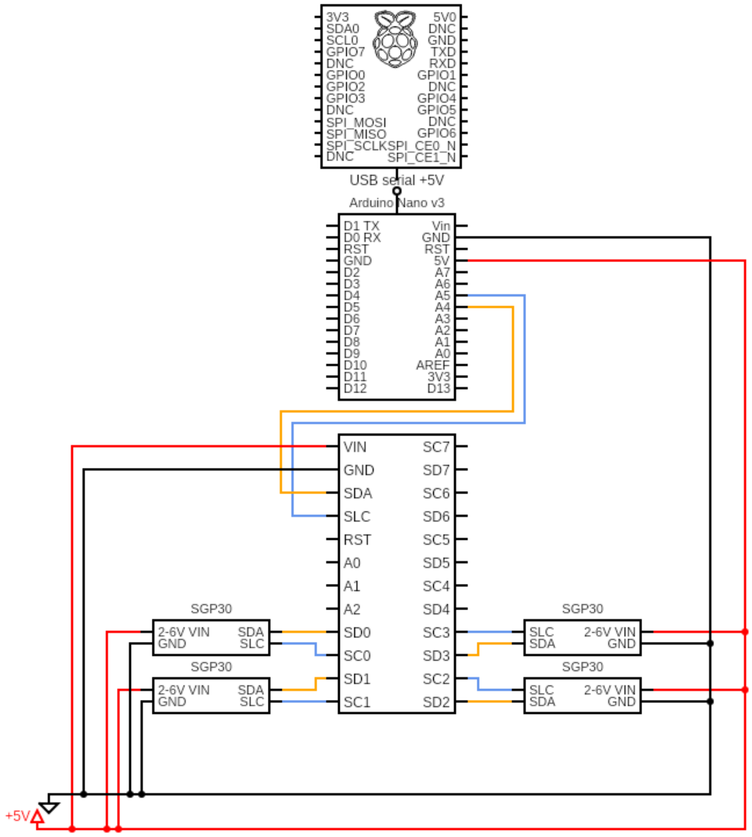
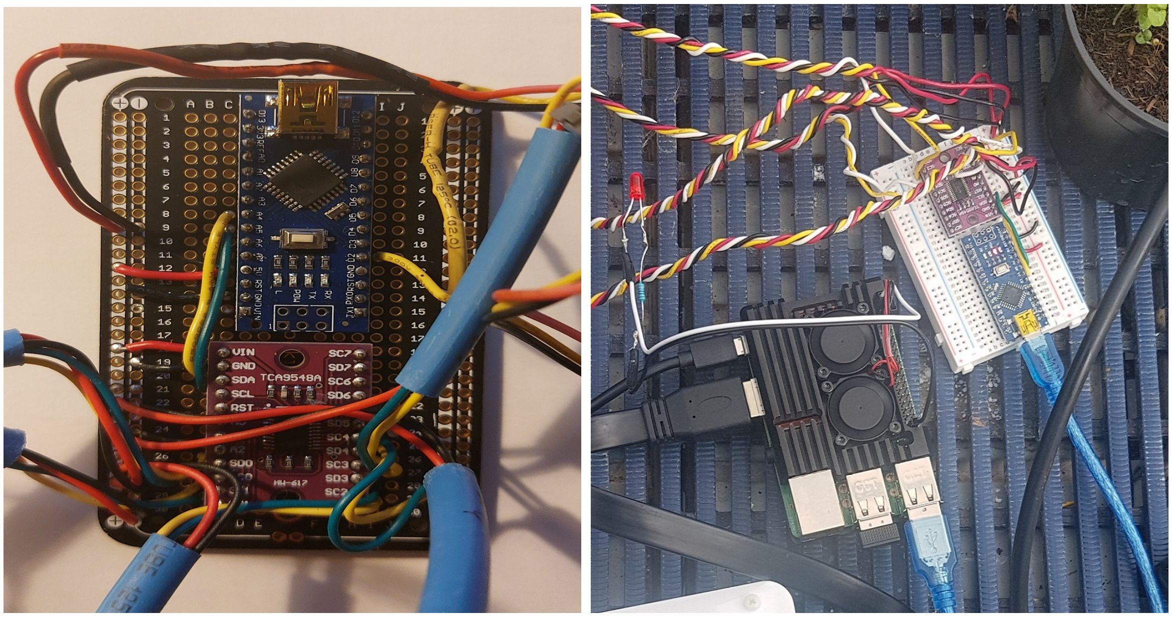


Figure 71: A simple circut diagram for the system orginally used in this experiment. Four SGP30 sensors are attached to various GPIO ports on the multiplexer, each one with a split I^{2}C channel. In the final model, a 5th sensor was added as a control. The multiplexer then relays information back the Arduino Nano, which processes the raw data from the sensors using the uploaded code into quantified data about the volatile compisition. This data is then communicated to the Raspberry Pi which time stamps and records it in an .CSV file.



(a)

(b)

Figure 72: Example of prototype bread-board configuration and Raspberry Pi 4, and PCB board fixed assessts on final model.

9.7 Code

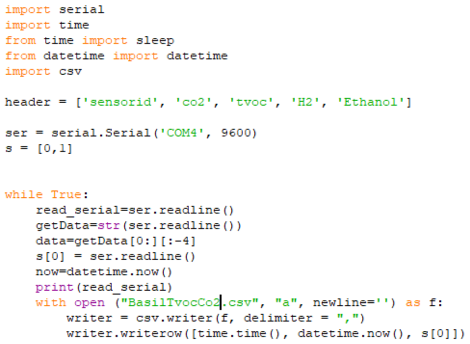
Calibrating the sensors required the use of a baseline measurement. This was achieved by running the sensors for a minimum of 12hrs in an inert environment before use and storing the baseline reading in the flash memory of the SGP30 (Sensirion 2018). Later trials using this technology (not shown here) used real-time calibration.

9.7.1 C/C++

The code for the SPG30 sensors and multiplexer were adapted from the ‘Sparkfun’ libraries to allow for multiple I^{2}C connections. Two versions of this code were used at different points in this study. At <5.1volt power availability, a humidity sensor (DHT22/AM2302) used to compensate for background interference would cause a power strain on the device, resulting in a loss of sensor information. This humidity sensor and code would provide a real-time *Baseline* level which results would be automatically calibrated to. Increasing accuracy by *ca.* 15 % (Sensirion 2018).

9.7.2 Python

The python code allows for direct writing to a .CSV file, thereby storing the data on the Pi for later processing.



```
import serial
import time
from time import sleep
from datetime import datetime
import csv

header = ['sensorid', 'co2', 'tvoc', 'H2', 'Ethanol']

ser = serial.Serial('COM4', 9600)
s = [0,1]


while True:
    read_serial=ser.readline()
    getData=str(ser.readline())
    data=getData[0:][:-4]
    s[0] = ser.readline()
    now=datetime.now()
    print(read_serial)
    with open ("BasilTvocCo2.csv", "a", newline='') as f:
        writer = csv.writer(f, delimiter = ",")
        writer.writerow([time.time(), datetime.now(), s[0]])
```

Figure 73: An example of the python script used to record data from the Adruino Nano. The import of the serial line allows for direct communication of data over the USB ports (COM4), and the recording of said data into a .CSV file with time stamping.

9.8 Results

9.8.1 Qualatative GC-MS

A Qualatative assessment of Coriander Volatiles was performed using gas chromotography, mass spectroscopy. This was performed in order to assess the potential phenolic compounds and demonstrate shifts in concentrations under various treatment effects.

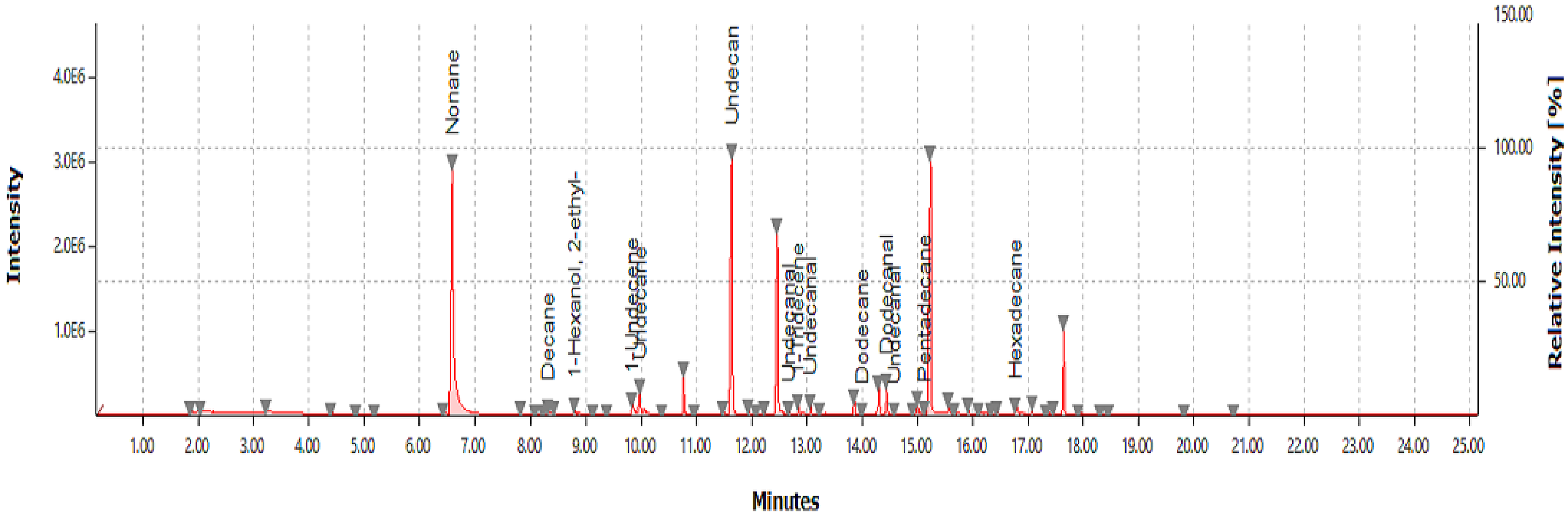


Figure 74: GC:MS readout.

Each substrate (Peat, Peat-Free, Peat-Reduced; not shown here) and treatment (AMF, Control) were assessed for volatile emissions (n=6 replicates) via SPME. Data was processed using openchrom software and open-source libraries were utilised for compound identifications. Total area of absorbtion was extrapolated for use in quantative analysis.

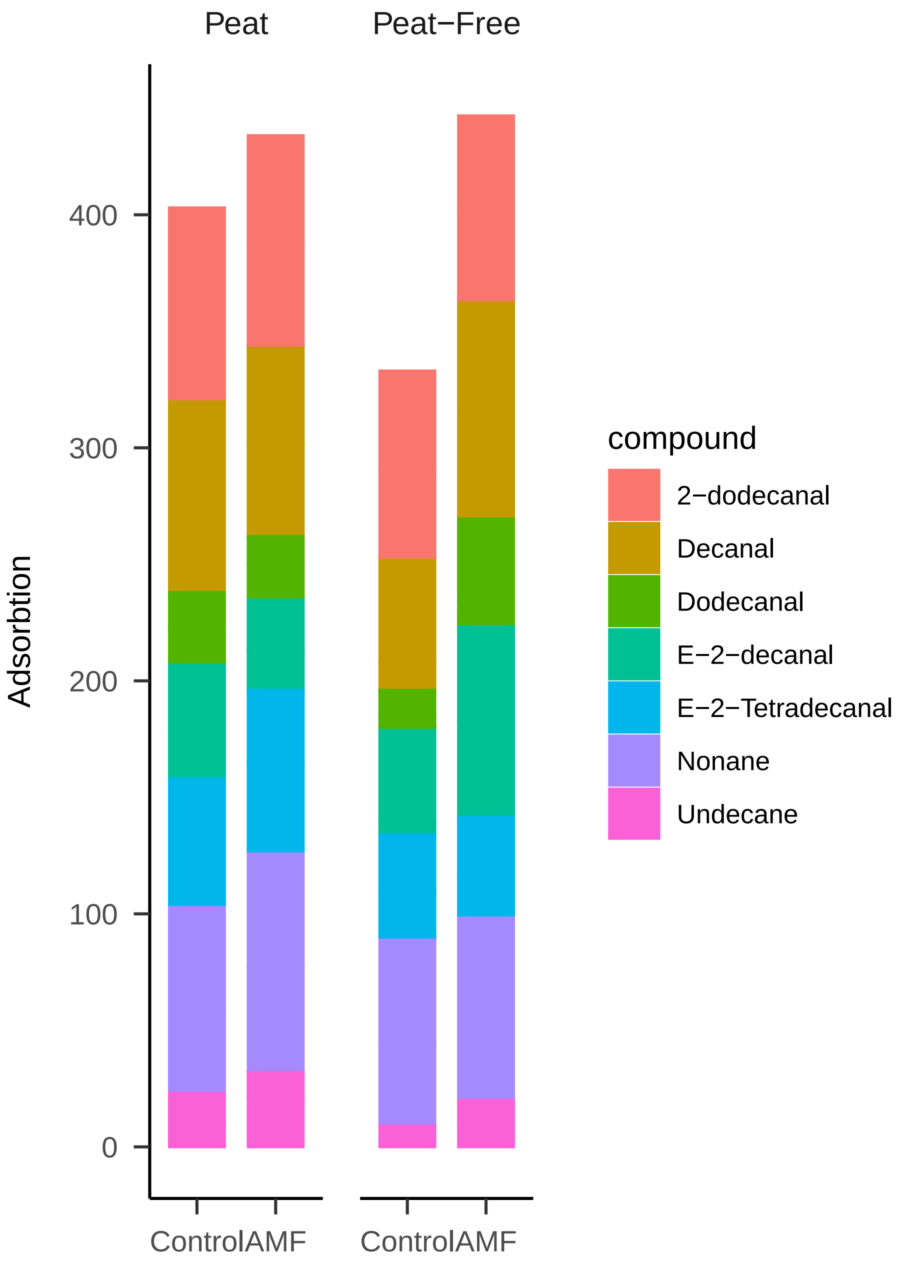


Figure 75: Quantified volatile compounds extrapolated from GC:MS. A significant increase in the abundance of Dodecanal can be seen in the Peat-Free substrates treated with AMF. This effect is not apparent in the Peat based substrates, even with AMF treatment. Peat-Free substrates appear to be subject to more significant change under AMF innoculation using plant volatile emissions as a proxy for treatment effect.

Highest mean adsoprtion values, and therefore highest emitter of Total volatile organic compound (TVOC) were substrates amended with AMF. The level of adsoprtion of TVOC's was for AMF was significantly higher than untreated substrates ($p < 0.05$), see Figure 75.

There was no significant difference between AMF treatments for either substrate. However a significant difference was demonstrated between Controls for each substrate, with Peat-Free substrates emitting significantly less TVOC comapre to Peat (201.3 and 166.5, respectively).

Table 35: Mean adsorbtion values for Compounds. * name shortened (+’*canal*’)

Subsbtrate	Trt	2-dode*	De*	Dode*	E-2-de*	E-2-Tetrade*	Nonane	Undecane	Total abs
Peat	Control	41.02	41.03	15.09	25.21	27.76	40.54	11.2	201.3
-	AMF	45.34	41.04	13.03	20.83	34.5	47.64	16.32	217.84
Peat-Free	Control	40.67	28.1	8.81	23.56	22.34	40.8	4.5	166.5
-	AMF	39.01	47.34	22.28	41.5	21.02	39.71	10.43	221.26

Table 36: Volatile Production

Peak	Volatiles Recorded	Potter et al, 1980
1	Nonane	nonane
2	3-menonane	octanal
3	decane	nonanal
4	4-hexen-1-0l, acetate	(E)-2-nonenal
5	2-hexanol	(Z)-4-decenal
6	1-undecene	9-decenal
7	undecane	decanal
8	?	(E)-2-decenal
9	decanal	l-decanol
10	4(prop-2-enyloxy) octane	10-undecenal
11	meundecane	undecanal
12	E-2-decenal	(E)-2-undecenal
13	S-dodecene	l-undecanol
14	undecanal	dodecanal
15	2-undecenal	(E)-2-dodecenal
16	?	tridecanal
17	dedecanal	(E)-2-tridecenal
18	2-dodecenal	tetradecanal
19	?	(E)-2-tetradecenal
20	2-dodecenal	pentadecanal
21	tetmedcane	
22	2,4-dodecndienal	
23	?-dodecenal	
24	pidodecanoate	
25	E-2-Tetnadecenal	

9.8.2 Real-Time plant gas monitoring

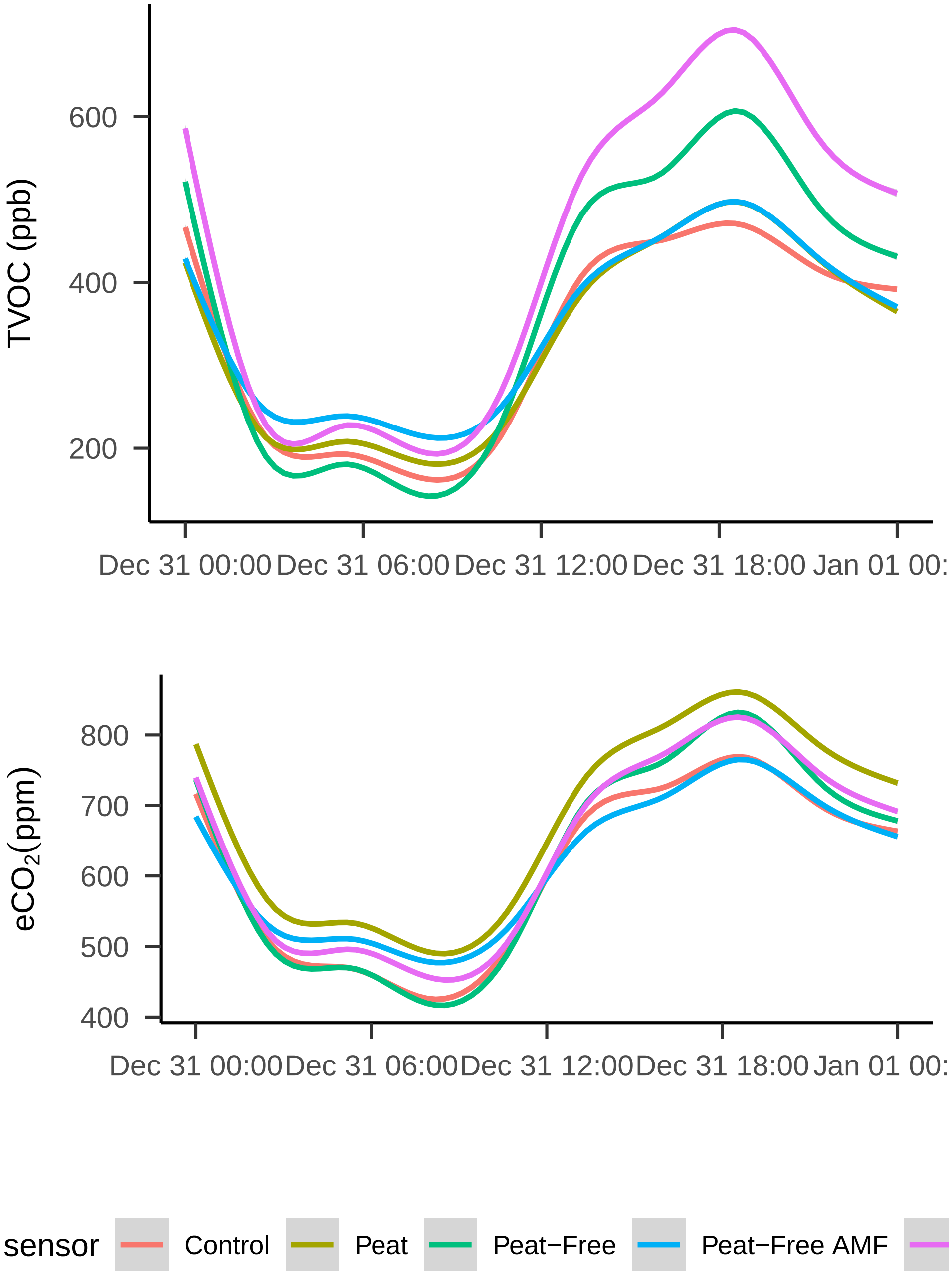


Figure 76: Randomly selected 24hour period of Total Volatile Compounds (TVOC) and estimated CO 2 (eCO 2) emissions detected by sensors. A clear cycle of emission concentrations in both gases can be seen over the 24hour period, with higher emissions occuring when light levels are at a peak (midday onwards).

Gas emissions over a 24hour period peak and trough alinged to time of day (Figure 76). An *ca.* difference of 40% change at peak emissions at 12 hour periods (06:00 and 18:00) may reflect peak Photosynthesis. Peat substrates demonstrate highest mean TVOC and CO_2 and the atmospheric control having the lowest emissions. The introduction of AMF appears to increase TVOC output in Peat substrates and reduce CO_2 emissions.

Table 37: 24 hour mean gas results

Sensor	mean TVOC	mean CO2
Control	326.18	604.67
Peat	330.32	674.14
Peat AMF	429.47	634.1
Peat-Free	366.83	620.46
Peat-Free AMF	345.34	615.43

9.8.3 40 Day observation of Plant gas emissions

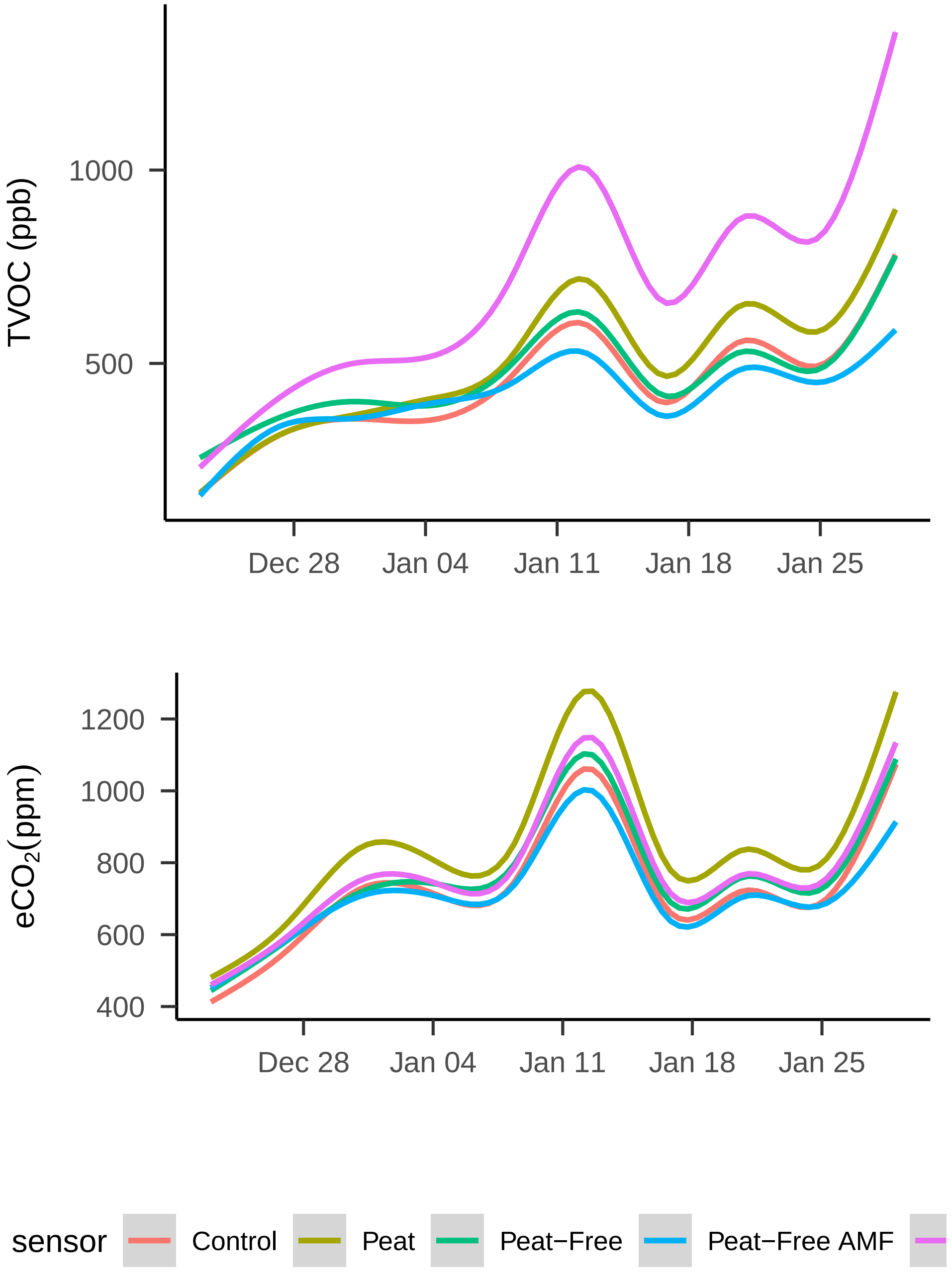


Figure 77: 40day gas emissions from Peat and Peat-Free pots, amended with AMF. The pattern of increasing and decreasing emissions is similar in both gases across all treatments. However the Peat AMF treatment appears significantly higher in emissions for TVOC throughout the entire time period, potentially suggesting these croppings were stressed.

Mean TVOC measurements (see Figure 77) for the entire trial period (n = 1,034,000) show Peat based substrates as the highest emittiers of gas. This is replicated in CO_2 emissions with higher emissions occuring from Peat (no AMF) substrate. Peat-Free substrates emit relatively less TVOC's over the trial period. Control values (atmopsheric) were similar to Peat-Free readings.

Table 38: 40day gas results

Sensor	mean TVOC	mean CO2
Control	435.3456	724.4517
Peat	493.6616	842.2432
Peat AMF	680.6818	772.5029
Peat-Free	459.528	754.9593
Peat-Free AMF	409.5001	710.1321

9.9 Discussion

Qualitative analysis (GC:MS) demonstrate significant treatment effects with the introduction of AMF in both Peat and Peat-Free substrates. This however did not replicate in continuous gas monitoring results. Throughout this trial period, Peat based substrates emitted higher levels of both Total Volatile Organic Compounds (TVOC) and CO2. This occurred for both 24hr period observation and for the total trial period. Higher levels of Total Volatiles may be indicative of stress. Typically volatile emissions such as aldehydes can be related to abiotic stresses such as drought or heat (Niinemets, Kännaste, and Copolovici 2013). Other work is indicative there is a potential dose-response to biotic activity such as pests or pathogens (Niinemets, Kännaste, and Copolovici 2013; Catini et al. 2019). There was no evidence of pest or pathogenic activity during the trial period, and no visible damage to croppings. This suggests the response in Volatile emissions was caused by abiotic/environmental stress. The irrigation system used during the trial was maintained to keep pots at 80% container capacity. Due to the automation of this system it is unlikely water stress was responsible. This is also true for light and heat stress due to the automatic environmental regulating system installed at the site. Higher CO2 emissions from Peat based pots over that of the Peat-Free pots may simply be due to properties of Peat as a growing media. Peat is a significant carbon sink (Barkham 1993), and therefore may continue to emit increased levels of CO2 when used as a growth medium. AMF are known for their ability to solubilise nutrients and exchange them with the host plant. This evidence of increased volatile emissions in Peat-AMF inoculated plants and decreased emissions in Peat Controls suggests the addition of AMF may have had a negative impact on plant quality, if using increased volatiles as a proxy for plant stress. Peat-Free substrates have demonstrated significant treatment effects when AMF is applied, in this instance; a reversal of decreased emissions in inoculated pots suggests a reduction in plant stress for Peat-Free media. Control values were higher than expected. Atmospheric measurements (Control) were taken <1m from the trial pots, and may have been influenced by proximity to not only trial pots, but other plants in the local environment. Treatment effects for both substrate and treatment (AMF) can be demonstrated through the observation of TVOC and CO2. However, further study should focus on monitoring emissions in sealed environments of single treatments. This will minimize impact of cross contamination from either treatments or plants. Further to this, the implementation of deliberate ‘Stress’ events such as pest introduction, drought or increased salinity may demonstrate the efficiency of monitoring TVOC and CO2 emissions for real-time plant health assessment.