Research Article

Received: 20 December 2023 Revised: 17 May 2024 Published online in Wiley Online Library:

(wileyonlinelibrary.com) DOI 10.1002/jsfa.13722

Peat-based amendment of soils reduces the complexity of the volatile profile in cultivated black truffles

Pedro Marco,[a,b*] Eva Tejedor-Calvo,[a,c] Ana Pilar Gracia,[b,d] Eva Gómez-Molina,[e] Sergi García-Barreda,[a,b] Sergio Sánchez[a,b] and M. Ángeles Sanz[b,f]

Abstract

BACKGROUND: Truffle cultivation is evolving rapidly and new agronomic practices such as 'truffle nests' (localized peat amendments of the orchard soil) are being developed. Truffle nests improve the shape of truffles and their depth in the soil and reduce the occurrence of insect damage but have also raised concerns about their impact on the ripeness and maturity of the harvested truffles. In this study, the effect of the nests on the volatile organic compounds profile and the aromatic profile of black truffles was evaluated, as well as the existence of perceptible sensorial differences in truffles. For this, truffles growing in nests were compared with truffles growing in the bulk soil of the same host tree.

RESULTS: Gas chromatography showed that nest truffles had a less complex volatile organic compound profile than bulk-soil truffles. Olfactometry indicated that nest truffles were associated with higher modified frequency values of odorants corresponding to sulfur-containing compounds. Despite this, sensory evaluation with consumers could not clearly show that nest truffles can be distinguished sensorially from bulk-soil truffles.

CONCLUSION: The results prove that soil conditions can influence the aromatic profile of truffles and thus suggest the possibility of managing truffle aroma using agronomic practices.

Supporting information may be found in the online version of this article.

Keywords: black truffle; volatile organic compounds; cultivation; aroma; truffle nests

INTRODUCTION

The European black truffle (*Tuber melanosporum* Vittad.) is a culinary delicacy, enjoyed worldwide due to its intense and unique aroma.[1] Truffles grow as obligate symbionts of several woody plants, mainly oaks, forming ectomycorrhizal relationships.[2] Cultivation of the truffle is a profitable activity for rural areas in southern Europe and truffle plantations are spreading to many countries even far from the natural range of the species.[3]

Truffle cultivation has evolved rapidly in recent decades, constantly incorporating new agronomic practices. One of these practices involves installing localized peat-based amendments, so-called 'truffle nests', in the soil, where truffle spores are added.[4,5] In this soft substrate, truffles grow free from disturbances caused by stones or by compacted soil. This leads to a rounder shape and lower occurrence of insect damage, which improves the market quality and value of truffles.[5,6] Truffle nests are very commonly used by Spanish growers, supported by several companies producing specialized substrates and *ad hoc* tractor implements.

* *Correspondence to: P Marco, Departamento de Ciencia Vegetal, Centro de Investigación y Tecnología Agroalimentaria de Aragón (CITA), Avda. Montañana, 50059 Zaragoza, Spain. E-mail: pmarco@unizar.es*

a *Departamento de Ciencia Vegetal, Centro de Investigación y Tecnología Agroalimentaria de Aragón (CITA), Zaragoza, Spain*

b *Instituto Agroalimentario de Aragón – IA2 (CITA-Universidad de Zaragoza), Zaragoza, Spain*

c *Department of Production and Characterization of Novel Foods, Institute of Food Science Research –CIAL (UAM–CSIC), Madrid, Spain*

d *Plant Food Research Group, Department of Food Technology, University of Zaragoza, Zaragoza, Spain*

e *Centro de Investigación y Experimentación en Truficultura (CIET), Diputación de Huesca, Graus, Spain*

f *Area de Laboratorios de Análisis y Asistencia Tecnológica, Centro de Investigación y Tecnología Agroalimentaria de Aragón (CITA), Zaragoza, Spain*

However, some truffle growers and traders have raised concerns about the impact of nests on truffle ripeness (development of its unique aroma) and maturity (development and melanization of spores, and resultant changes in gleba color and structure). The aroma is the primary quality responsible for the gastronomic value of truffles, and it can be influenced by the ascocarp maturity stage, the host tree, and soil microbial communities.[7-9]

Despite the gastronomic importance of truffle aroma, markets rarely take it into account when setting truffle price (except for the presence of a foreign smell and/or taste),[6] due to the difficulties in obtaining rapid, quantitative, objective measurements of aroma. The black truffle is characterized by a complex aroma made of a mixture of about 100 volatile organic compounds (VOCs), among which the most important are butane-2,3-dione (diacetyl), dimethyl disulfide (DMDS), ethyl butanoate, dimethyl sulfide (DMS), 3-methylbutan-1-ol, and 3-ethyl-5-methylphenol.[7,10,11]

A previous study found that the truffle nest technique did not affect the maturity of truffles significantly, with nest truffles and bulk-soil truffles harvested on the same date showing the same spore maturity index.[5] On the other hand, truffles growing in nests were more common in the early harvesting season, when maturity was lower, and truffle nests more frequently produced ascocarp clusters, which could present truffles at different maturity stages. On the other hand, truffles in nests develop surrounded by a peat-based substrate, presenting different physico-chemical properties and microbiota that could affect the truffle metabolism and aroma development.[9,12]

The aim of this study was to investigate whether the agronomic practice of truffle nests affects black truffle aroma. For this, a comparison was made between the VOC profile and aromatic profile of truffles growing in truffle nests and those growing in bulk soil. Potential markers for discriminating truffles grown in truffle nests were sought. Finally, sensorial tests were employed to assess the existence of perceptible sensorial differences between nest truffles and bulk-soil truffles.

MATERIAL AND METHODS

Study site and experimental design

Black truffle ascocarps were harvested in an 18 year-old orchard of holm oaks (*Quercus ilex* L.) in Teruel province (Spain). All the plantation is managed in the same way, but it is divided into two zones with different soil types: ST1 with a sandy loam texture and ST2 with loam texture (Supporting Information, Table S1). The orchard owner had been setting up truffle nests in all the trees every year during the previous 6 years and, consequently, he harvested numerous truffles from nests and from the bulk soil every year. The installation of the nests involved digging holes about 25 cm deep, filling them with 1.5 L of a *Sphagnum* peat-based substrate (Turbatruf from Projar, Valencia, Spain) a black peat – white peat – coir – perlite mix 11:5:3:1, with pH raised to 7.5; Supporting Information, Table S2) and re-covering the substrate with soil. Ground ripe truffle ascocarps (0.1 g dry ascocarp per liter of substrate) were mixed thoroughly with the substrate before being used in the field. The nests were set up in the *brûlé*, the soil around the host tree where most truffles are produced.[5]

Three trees were selected randomly in each soil type. In each tree, two truffles were harvested: one growing in a nest and the other one in the bulk soil. The total sample size was 12 truffles. All the truffles were harvested on the same date (7 March 2017), in the late season when truffles typically show high levels of ripeness and maturity.[5,7] The truffles were localized with the aid of a trained dog, the same one for all truffles. Sometimes, when digging the spot marked by the dog, several truffles growing in close proximity were found (ascocarp clusters). These were discarded to avoid potential bias linked to variability in maturity.[5] Only truffles that grew alone, separately from other ones, were included in the study. Truffles with abiotic or biotic damages (resulting in rotting, softened texture or galleries) were also discarded. Truffles weighing less than 15 g were also discarded to avoid subsequent methodological problems, thus the sampled truffles weighed 15–40 g.

In the lab, every truffle was cleaned by gently brushing under fresh tap water and 15 min of ultrasound treatment. Then truffles were surface air dried and cooled at 7 °C on separate containers until further analysis. The maturity stage of the truffles was assessed with a gleba sample reaching 5–10 mm under the peridium, taken with a scalpel. With this sample, a spore maturity index was calculated as the percentage of asci containing mature (i.e., dark brown and spiny) spores.[13]

Headspace gas chromatography–mass spectrometry analysis

The aromatic profile was analyzed using the static headspace (HS) technique with a Turbomatrix HS16 sampler (PerkinElmer, Hopkinton, MA, USA) modifying conditions from a previous work.[7] Four grams of each truffle sample were sliced finely (so that they were around 1 mm thick) and placed in a 20 mL glass vial, which was hermetically sealed with a septum. The headspace was programmed to 120 °C for 15 min and 1 min of pressurization time. The injection was carried out over 6 s at 20 psi and the inlet temperature was 220 °C. The HS sampler was connected to a Clarus 500 gas chromatography system coupled with a mass spectrometer (PerkinElmer) equipped with a DB-wax capillary column (60 m × 0.25 mm i.d. × 0.25 μm film thickness) (Agilent Technologies, Santa Clara, CA, USA). A flow of 1 mL min^{-1} was used with helium as a carrier gas. The oven temperature was 45 °C held for 2 min, 45–110 °C at a rate of 7 °C min^{-1}, and finally to 225 °C at 25 °C min^{-1}, and held for 5 min. The mass spectrometer used the electron impact (EI) mode with an ionization potential of 70 eV and an ion source temperature of 200 °C. The interface temperature was 220 °C. The mass spectrometer scanning was recorded in full scan mode (35–300 m/z). TurboMass ver. 5.4.2 software was used to control the GC–MS system. Peak identification of the volatile components was achieved by comparison of the mass spectra with mass spectral data from the National Institute of Standards and Technology (NIST) MS Search Program 2.0 library and by comparison of the previously reported retention index (RI) with those calculated using an *n*-alkane (C6-C20) series under the same analysis conditions. The VOCs were semiquantified by integrating the area of one ion characteristic of each compound and normalization by calculating the relative percentage.

Gas chromatography–olfactometry analysis

A solid-phase microextraction (SPME) holder (Supelco, Bellefonte, PA, USA) was used to perform these experiments. A fiber of medium polarity was used to avoid discrimination toward very nonpolar and polar volatile compounds. A fused silica fiber coated with a 50/30 μm layer of divinylbenzene/carboxen/polydimethylsiloxane from Supelco (Barcelona, Spain) was therefore chosen to extract the aromatic compounds. As optimized in a previous work,[1] 2 g of truffle finely sliced (around 2 mm thick) was placed in a 20 mL glass vial closed with a septum. Once the desired temperature (53 °C) had been reached, the vial was conditioned at 53 °C for 5 min. After this time, the fiber was introduced into the

vial and exposed to the headspace of the truffle for 13.6 min. The gas chromatography–olfactometry (GC-O) analysis was carried out in a gas chromatograph HP 4890 (Termoquest, Milan, Italy) with a flame ionization detector (FID) and an olfactometric port ODO-I from SGE (Ringwood, Australia). This instrument was equipped with a DB-WAX (polyethylene glycol) capillary column from J&W Scientific (Folsom, CA, USA), 30 m, 0.32 mm i.d., 0.5 μm film thickness, and a pre-column (3 m; 0.32 mm i.d.) from Supelco. Chromatographic conditions were as follows: nitrogen as the carrier gas (3.5 mL/min); splitless injection (splitless time 60 s); injector and detector temperature 220 °C. The oven temperature program was the following: 40 °C for 5 min, then raised at 6 °C min^{-1} to 220 °C, this temperature was held for 15 min for cleaning purposes.

A panel of three judges carried out the sniffing of the extract. Sniffing time was approximately 40 min, and each judge conducted two sniffs per day (by tree, nest truffle and bulk-soil truffle). The panelists were asked to score the intensity of each aromatic stimulus using a seven-point scale (0 = not detected; 1 = weak; 2 = clear but not intense note; 3 = intense note; intermediate values 0.5, 1.5 and 2.5 being allowed). The signal obtained was modified frequency (MF), a parameter that was calculated with the formula: $MF(\%) = [F(\%) \times I(\%)]^{0.5}$, where F(%) is the detection frequency of an aromatic attribute expressed as a percentage of the total number of judges, and I(%) is the average intensity expressed as a percentage of the maximum intensity.[14] The identification of the odorants was carried out by a comparison of their odors, chromatographic retention index in the DB-WAX column with those of pure reference compounds. The standards used for identifications were supplied by Aldrich (Steinheim, Germany), Fluka (Buchs, Switzerland), PolyScience (Niles, IL, USA), Lancaster (Strasbourg, France), Alfa Aesar (Karlsruhe, Germany).

Sensorial test: blind-olfactory triangle test

To evaluate the existence of perceptible sensorial differences between nest truffles and bulk-soil truffles, a discriminative sensory evaluation was conducted through 302 blind-olfactory triangle tests of truffle consumers, using the ISO 4120:2021 methodology for triangular tests.[15] The panel, comprising 30 people who had previously come into contact with truffles as consumers, was recruited from the staff and students of Agrifood Research and Technology Centre of Aragón and the University of Zaragoza. Six tests were performed, each using one nest truffle and one bulk-soil truffle harvested in the same tree. Each truffle ascocarp was cut into three pieces using a sharp knife and each piece was placed into a different canister. For each pair of truffles, the subjects were asked to smell two sets of three unidentified samples (the first set with one nest sample and two bulk-soil samples, and the second one the other way round) and judge which sample had a different odor in each set. Truffles were covered during the experiment to avoid visual perception. Each pair of truffles was evaluated in a separate session, from 9 to 16 March 2017.

Statistical analyses

Principal component analysis (PCA) was used to explore the variability in VOC profiles identified by headspace gas chromatography–mass spectrometry (HS-GC–MS) and aromatic profiles identified by GC-O. In particular, we analyzed the differences between truffles growing in nests and those growing in the bulk soil. Analysis of variance (ANOVA) was used to evaluate the differences in the number of VOCs identified in the HS-GC–MS and the number of odor zones detected during the GC-O, with a 95% confidence level ($\alpha = 0.05$). The assumptions for ANOVA (homogeneity of variance and normality) were checked. The results of the sensory triangle tests were evaluated with binomial test tables. All the statistical analyses were performed with the statistical software R (R Core Team 2019, Vienna, Austria).

RESULTS

Spore maturity

All the truffles showed spore maturity indices higher than 0.7, without significant differences between nest truffles and bulk-soil truffles (mean values: 0.78 and 0.83 respectively, $t = -1.0$, $P = 0.33$).

VOC profile

Thirty-seven VOCs were identified by HS-GC–MS, with soil-bulk truffles showing significantly more VOCs (mean: 31, standard deviation SD: 3) than nest truffles (mean: 20, SD: 3, $F = 43.9$, $P < 0.001$). Only nine of the VOCs showed a mean area percentage higher than 2.5%. These were alcohols (2-methylbutan-1-ol, pent-4-en-2-ol and 2-methylpropan-1-ol), aldehydes (2-methylbutanal and ethanal), esters (2-methylpropyl 2-methylpropanoate and prop-1-en-2-yl acetate), and sulfur-containing compounds (carbon disulfide and DMS). All these compounds appeared in all samples except one (Table 1).

The PCA of the HS-GC–MS data allowed, with the first principal component (PC1), the clear separation of the nest truffles from bulk-soil truffles, with the former showing a much narrower range for PC1 scores (Fig. 1). The PC1 explained 28.9% of the variability in the samples. However, among the more common compounds (mean percentage of area higher than 2.5%), only the ester prop-1-en-2-yl acetate showed a strong loading value for PC1 (which was positive). Many compounds that occurred less frequently showed strong positive loading values for PC1, including alcohols (butan-2-ol, hexan-2-ol, octan-3-ol), aldehydes (butanal, heptanal, octanal), esters (2-methylpropyl 2-methylbutanoate, 3-methylbutyl 2-methylbutanoate), ketones (pentan-2-one, pentane-2,3-dione, pent-3-en-2-one), and the aromatic compound methoxybenzene (anisole) (Fig. 1). These compounds were associated with the bulk soil. On the other hand, no compound showed strong negative loading values for PC1, which would be associated with nests (Fig. 1, Supporting Information, Fig. S1(a)).

The second principal component (PC2) explained 20.9% of the variability in the samples. Several alcohols (2-methylpropan-1-ol, pentan-2-ol, pent-1-en-3-ol) showed strong positive loading values for PC2, whereas several other compounds showed strong negative loading values for PC2: an alcohol (propan-1-ol), aldehydes (ethanal and 2-methylbutanal), and a ketone (butan-2-one) (Fig. 1).

Aromatic profile

Twenty-one different odor zones were detected by GC-O, with no significant differences in the number of odor zones between truffles grown in the bulk soil (mean: 14, SD: 2) and those in nests (mean: 15, SD: 2, $F = 2.3$, $P = 0.16$). The odorants with higher modified frequency (MF) values corresponded with ethyl 2-methylbutanoate (strawberry odor), oct-1-en-3-one (mushroom odor), 3-(methylsulfanyl)propanal (methional - baked potato odor), octanal (citric odor), DMS (black olive/truffle odor), hexanal (green odor), DMDS (truffle odor), and an unidentified (truffle odor) compound with retention time (RT) 3.11 min (Table 2).

The first principal component of the GC-O data distinctly separated nest truffles from bulk-soil truffles, despite some overlap

Journal of The Science of Food and Agriculture published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry.

Table 1. Volatile compounds identified in black truffle samples and related percentages of area values (%) obtained using the headspace gas chromatography–mass spectrometry (HS-GC–MS) analysis

Number	RT (min)	Identity (IUPAC)[a]	CAS Number	Percentage of area											
				N1.1	N1.2	N1.3	N2.1	N2.2	N2.3	BS1.1	BS1.2	BS1.3	BS2.1	BS2.2	BS2.3
1	4.13	Hexane	110–54-3	-	1.71	0.96	1.06	-	2.40	-	1.49	0.90	3.19	3.60	1.42
2	4.21	Heptane	142.82–5	-	1.34	0.68	12.83	-	2.14	3.86	1.82	-	0.41	2.31	-
3	4.41	Carbon disulfide	75–15-0	3.30	2.06	9.40	4.46	7.24	4.49	1.71	3.33	1.27	1.17	4.38	1.12
4	4.46	Methanethiol	74–93-1	5.07	-	-	-	-	-	-	-	-	-	-	-
5	4.80	Ethanal	75–07-0	2.37	9.10	12.96	2.80	2.91	4.92	7.68	1.66	10.66	9.32	2.89	9.41
6	5.19	Dimethyl sulfide (DMS)	75–18-3	2.36	4.94	2.21	2.53	2.87	1.16	2.02	-	2.72	4.63	3.07	2.40
7	5.54	Ethanethiol	75–08-1	-	0.02	1.39	0.04	0.11	0.21	0.20	0.21	0.46	0.81	0.20	0.41
8	5.90	Prop-1-en-2-yl acetate	108–22-5	3.70	5.05	2.38	0.86	3.22	1.93	3.41	7.45	6.23	7.14	4.91	5.50
9	6.19	Butanal	123–72-9	-	-	-	-	-	-	-	0.01	0.01	-	-	0.01
10	7.27	Butan-2-one	78–93-3	0.20	0.44	0.33	0.13	-	0.19	0.33	0.99	0.54	1.19	0.25	0.48
11	7.54	2-Methylbutanal	96–17-3	2.74	9.84	5.24	2.34	11.48	2.38	4.42	7.47	9.29	23.10	6.79	8.20
12	8.00	Pent-4-en-2-ol	625–31-0	7.99	20.00	-	13.98	10.10	16.29	30.00	25.66	25.01	17.54	10.90	22.08
13	8.30	Hexan-2-ol	626–93-7	0.06	-	-	-	-	0.04	0.07	0.20	0.26	0.06	0.48	0.23
14	8.88	Pentan-2-one	107–87-9	-	-	-	-	-	-	-	0.01	-	-	-	-
15	10.16	Butan-2-ol	78–92-2	-	-	-	-	-	-	0.20	0.38	0.32	0.25	0.14	0.28
16	10.49	Pentanal	110–62-3	0.24	-	0.11	0.06	-	-	-	-	0.02	-	0.00	0.02
17	10.53	Butane-2,3-dione	431–03-8	0.08	-	-	0.05	0.20	-	0.03	-	-	-	0.09	-
18	10.53	Propan-1-ol	71–23-8	0.06	1.46	2.49	0.18	0.39	1.24	3.37	3.26	3.80	4.15	1.42	3.36
19	10.67	Ethyl 3-methylbutanoate	108–64-5	-	-	-	0.12	0.03	-	-	-	-	-	0.02	-
20	10.91	Ethyl 2-methylbutanoate	7452-79-1	0.35	-	0.84	0.48	0.08	0.46	0.20	0.43	0.27	0.17	0.44	0.24
21	10.97	Pentane-2,3-dione	600–14-6	-	-	0.33	0.11	0.29	-	0.15	0.37	0.48	0.10	1.07	0.43
22	11.60	Dimethyl disulfide (DMDS)	624–92-0	0.05	-	0.24	-	-	0.33	-	0.04	0.04	0.39	0.07	0.03
23	11.70	Hexanal	66–25-1	0.04	-	0.24	-	-	0.33	0.07	0.11	0.13	0.39	0.23	0.11
24	11.93	2-methylpropyl 2-methylpropanoate	97–85-8	25.73	-	13.71	0.04	12.57	17.06	0.01	0.01	13.62	5.68	0.07	-
25	12.09	2-methyl-propan-1-ol.	78–83-1	25.73	8.98	13.71	13.25	12.57	17.06	9.08	18.32	10.80	5.68	21.70	13.89
26	12.83	Pentan-2-ol	6032-29-7	-	-	-	0.03	-	-	-	-	-	-	0.03	-
27	13.05	(*E*)-Pent-3-en-2-one	3102-33-8	-	-	-	0.03	-	-	0.03	0.01	0.08	-	0.11	0.04
28	14.32	Pent-1-en-3-ol	616–25-1	0.06	-	-	-	-	-	0.02	0.04	-	-	0.06	-
29	14.44	2-methylpropyl 2-methylbutanoate	2445-97-2	0.03	-	-	-	-	0.05	0.03	0.13	0.04	0.02	0.19	0.07
30	14.99	Heptanal	111–71-7	-	-	-	-	-	-	-	0.06	0.01	-	0.08	0.03
31	15.20	2-Methylbutan-1-ol	137–32-6	19.78	35.03	32.78	44.56	35.92	27.27	32.91	26.34	12.95	14.51	34.03	30.07
32	15.29	Octanal	124–13-0	-	-	-	-	-	-	-	0.02	-	-	0.03	-
33	17.46	3-methylbutyl 2-methylbutanoate	27 625–35-0	-	-	-	0.04	-	0.05	0.14	0.15	0.04	0.04	0.21	0.13
34	19.29	Methoxybenzene	100–66-3	-	-	-	-	-	-	0.01	0.01	0.01	0.02	0.03	0.01
35	19.42	Octan-3-ol	589–98-0	-	-	-	-	-	-	0.01	0.03	0.01	0.01	0.09	0.01
36	22.07	1-methoxy-3-methylbenzene	100–84-5	0.04	-	-	-	-	-	-	-	-	-	0.02	-
37	24.24	1-ethyl-4-methoxybenzene	1515-95-3	-	-	-	0.03	-	-	-	-	-	-	-	-

[a] Tentative identification based on comparison of mass spectra with mass spectral data from the National Institute of Standards and Technology (NIST) MS Search Program 2.0 library, and by comparison of previously reported retention index with those calculated using an *n*-alkane series. The samples are named N for nest and BS for bulk soil, with the first number indicating the soil type (1, 2) and the second number specifying the truffle ID (1–3). RT, retention time; - indicates not detected (raw area value below 500).

due to the broader range of PC1 scores in bulk-soil truffles (Fig. 2). The first principal component explained 23.1% of the variability in the samples, with odorants linked to sulfur-containing compounds (DMS, DMDS), butane-2,3-dione and 4-hydroxy-2,-5-dimethyl-3-furanone (furaneol) showing strong positive loading values for PC1. These compounds were associated with truffles growing in nests. The odorants linked with (3Z)-hex-3-enal and butanoic acid showed strong negative loading values for PC1, associated with truffles growing in the bulk soil (Fig. 2).

The second principal component explained 17.3% of the variability in the samples. The odorant linked to phenethyl acetate showed a strong positive loading value for PC2, whereas the

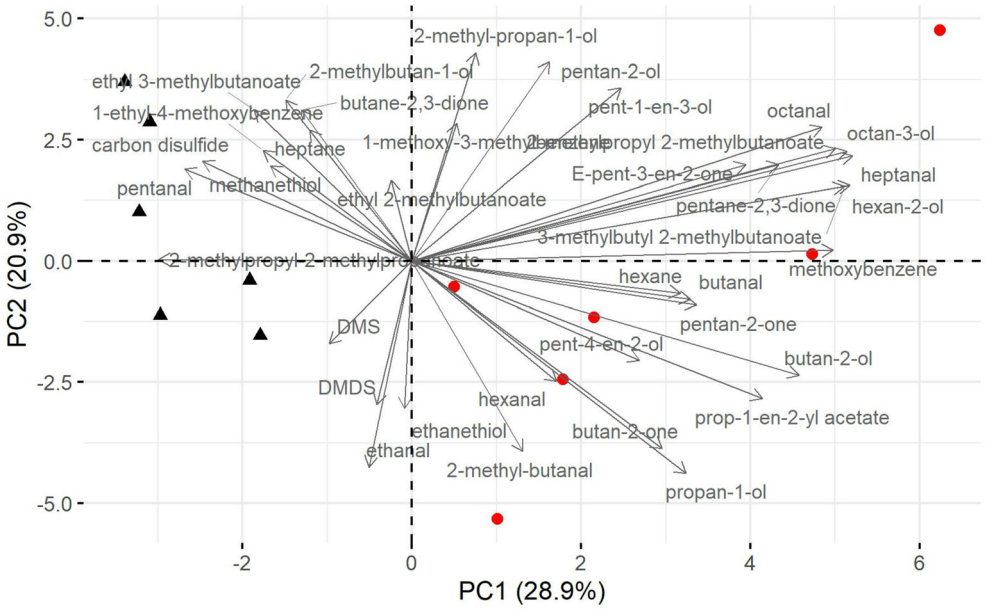

Figure 1. Principal component analysis (PCA) biplot for the (scaled) percentage of area values of the volatile organic compounds (VOCs) detected by headspace gas chromatography–mass spectrometry (HS-GC–MS) in truffles growing in bulk soil (red dots) and nests (black triangles). The VOCs are listed in Table 1.

odorants linked to an unidentified compound (RT 3.11 min) and ethyl-butanoate showed a strong negative loading value for PC2 (Fig. 2).

Sensory evaluation

The triangle tests carried out in six pairs of truffles (bulk soil vs nest) delivered relatively unclear results. Consumers perceived nest truffles as different from bulk-soil truffles in two of the tests in ST2, whereas for ST1 they only perceived nest truffles as different in one of the three tests (Table 3). This is in line with the PC1 scores for the aromatic profiles, which show that bulk-soil truffles of ST1 overlap with nest truffles whereas soil-bulk truffles of ST2 do not (Supporting Information, Fig. S1(b)).

DISCUSSION

The results show that black truffles grown in nests exhibit a much less complex VOC profile, with fewer components than truffles growing in the bulk soil (13–23 compounds compared to 24–33). However, among the VOCs with higher occurrence, only one (prop-1-en-2-yl acetate) showed a strong association with the bulk soil, with no VOC showing a strong association with nests. This is the first investigation to report a distinct effect of edaphic conditions on the VOC profile of black truffles. The importance of this finding is supported by the fact that the experimental design controlled for the major factors influencing the VOC profile: the maturity stage of the ascocarp, the date of harvesting, and the occurrence of abiotic and biotic damages.[5,7,16,17] This could open the way for investigating the role of edaphic variability on the aromatic profile of truffles and the possibilities to manage it agronomically.

However, it must be considered that the properties of peat moss as a substrate are substantially different from those of mineral soils, both chemically – with peat being more than 90% organic matter and showing lower nutrients content – and physically – with higher aeration and drainability, higher water retention, and easily available water, and lower thermal conductivity under equal water content.[18-20] The microbiological populations, which could play a role in black truffle aroma,[9] are very likely to be different too,[21] because *Sphagnum* peat moss is usually imported from boglands with anaerobic conditions in northern Europe. All these factors could be responsible for the alteration of the VOC profile. For instance, it was found previously that the apparent density of truffle ascocarps fluctuated more in nest truffles than in bulk-soil truffles, thus suggesting that truffles growing in nests were subject to sharper fluctuations in water content during their late stages.[5] This could influence their metabolism and thus aroma development.[12]

Nest truffles were strongly associated with relatively higher levels of the sulfur-containing compounds, DMS and DMDS. Early season (December–January) truffles were characterized by higher levels of DMS and DMDS, whereas late-season (February–March) truffles were characterized by higher levels of carboxylic acids such as butanoic acid.[7] This suggests that the aromatic profile of nest truffles has more in common with truffles in early season ripening stages than would be expected for truffles harvested in late season (March). In contrast, the spore maturity index was not altered in the nests, this highlighting the complex relationship between maturity and ripeness in truffles.[5,22]

The sensory evaluation did not show clearly that truffle consumers can sensorially distinguish nest truffles from bulk-soil truffles – this would require a higher discrimination ability. Interestingly, for ST2 both the GC–O and the sensory panel showed higher ability to discriminate between nest truffles from bulk-soil truffles, when compared to ST1. This suggests that in some soils the truffle aroma is more similar to nest truffles than in others, although these differences between soil types did not appear in the VOC profile.

Finally, the lack of clear differences in the sensory evaluation suggests that the reported issues with maturity and ripeness in nests could be related mostly to ascocarp clusters (clusters of truffles growing in close proximity). When a dog marks one of this

Journal of The Science of Food and Agriculture published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry.

Table 2. List of odor compounds identified by GC–O analysis: retention time (RT), chemical identity, CAS number, odor descriptor, linear retention index (LRI), and modified frequency (%)

Num	RT (min)	Identity (IUPAC)	CAS number	Odor descriptor	LRI DB-WAX	N1.1	Modified frequency N1.2	N1.3	N2.1	N2.2	N2.3	BS1.1	BS1.2	BS1.3	BS2.1	BS2.2	BS2.3
1	3.11	ni	-	Truffle 1	<1000	67	83	69	83	65	67	81	83	64	63	66	67
2	3.58	Dimethyl sulfide (DMS)[a]	78–18-3	Truffle 2, Black olive	943	83	58	83	85	88	83	53	83	92	52	50	56
3	6.07	Dimethyl disulfide (DMDS)[a]	624–92-0	Truffle 3	-	67	83	50	81	83	88	66	83	87	-	-	-
4	8.02	Butane-2,3-dione[a]	431-03-8	Strawberry/ Dairy	988	50	-	53	31	54	-	52	67	-	-	-	-
5	10.09	Ethyl butanoate[b]	105–54-4	Dairy	1033	-	-	48	54	-	-	42	50	-	-	-	52
6	10.45	Ethyl 2-methylbutanoate[a]	7452-79-1	Strawberry 1	1052	92	92	92	87	92	96	93	83	83	85	84	85
7	11.06	Ethyl ethanoate[b]	141–78-6	Strawberry/ Pineapple	1063	-	50	83	67	32	31	51	33	-	53	53	50
8	11.49	Hexanal[b]	66-25-1	Green	1086	58	58	67	33	67	66	63	58	59	69	66	69
9	13.53	(3Z)-hex-3-enal[b]	6789-80-6	Strawberry 2	1147	-	58	-	-	-	-	59	-	43	51	51	-
10	16.13	3-methylbutan-1-ol[b]	123-51-3	Bitter	1214	-	67	67	49	83	85	-	67	66	50	55	-
11	19.04	Octanal[b]	124-13-0	Citric	1132	92	83	92	86	92	92	-	83	67	96	53	90
12	19.32	Oct-1-en-3-one[a]	4312-99-6	Mushroom	1302	83	98	98	83	83	83	81	83	100	84	83	87
13	20.52	1-(3,4-Dihydro-2H-pyrrol-5-yl) ethan-1-one[a]	99 583-29-6	Popcorn	1360	67	67	52	-	-	-	-	-	-	-	-	-
14	21.54	(5Z)-octa-1,5-dien-3-one[b]	65 767-22-8	Geranium	1394	33	0	53	52	61	57	65	50	52	63	66	67
15	23.56	Ethanoic acid[a]	64–19-7	Vinegar	1463	50	50	-	-	50	-	37	50	-	39	33	50
16	24.26	3-(methylsulfanyl)propanal[a]	3268-49-3	Baked potato	1481	83	83	95	58	65	69	41	83	81	92	92	93
17	29.30	Butanoic acid[b]	107–92-6	Cheese	1669	-	-	-	-	-	-	-	-	-	50	55	-
18	31.57	(2E,4E)-nona-2,4-dienal[a]	5910-87-2	Legume 1	1767	50	67	33	50	52	53	-	-	-	42	47	-
19	34.02	(2E,4E)-deca-2,4-dienal[b]	2363-88-4	Legume 2	1855	-	58	48	70	-	-	-	67	-	52	50	-
20	34.59	2-Phenylethyl acetate[b]	103–45-7	Flower	1895	58	-	51	-	67	67	-	-	-	-	-	-
21	42.48	4-Hydroxy-2,5-dimethyl-3-furanone[b]	3658-77-3	Burnt caramel	1971	-	-	71	67	58	-	-	50	-	-	-	-

[a] Identification was based on matching gas chromatographic retention times with those of pure compounds available in the laboratory.
[b] Tentative identification was based on comparison with linear retention index (LRI) databases published in the literature.
The samples are named N for nest and BS for bulk soil, with the first number indicating the soil type (1, 2) and the second number the truffle ID (1–3); ni, not identified.

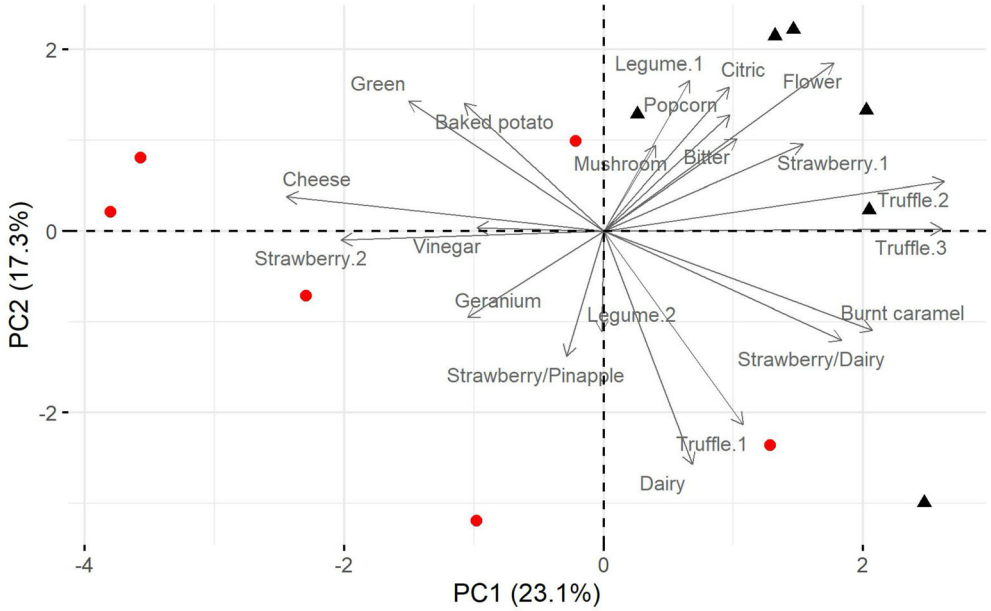

Figure 2. Principal component analysis (PCA) biplot for the (scaled) modified frequency of the odor attributes detected by gas chromatography–olfactometry (CG-O) in truffles growing in bulk soil (red dots) and nests (black triangles). Odor descriptors are listed in Table 2.

Table 3. Sensory triangle tests carried out with consumers for discriminating the aroma of nest truffles from that of bulk-soil truffles in two different soils (carried out according to the standard ISO 4120)

Sensory discrimination triangle test	Answers (correct/total)	*P*
Soil type 1, tree 1.1: bulk-soil versus nest	28/46	**<0.001**
Soil type 1, tree 1.2: bulk-soil versus nest	18/56	0.62
Soil type 1, tree 1.3: bulk-soil versus nest	12/44	0.84
Soil type 2, tree 2.1: bulk-soil versus nest	20/46	0.098
Soil type 2, tree 2.2: bulk-soil versus nest	28/50	**<0.001**
Soil type 2, tree 2.3: bulk-soil versus nest	31/60	**0.003**

Significant differences ($\alpha = 0.05$) in bold.

clusters, it is only indicating that at least one ascocarp is ripe. Considering that nests increase the occurrence of these clusters, it would be interesting to investigate their effect on maturity and ripeness.[5]

To conclude, black truffles grown in nests exhibited a much less complex VOC profile, much poorer in compounds, than truffles growing in the bulk soil. Truffles growing in nests showed a strong association with odorants corresponding to sulfur-containing compounds. However, despite these differences, sensory evaluation with truffle consumers could not show clearly that nest truffles can be sensorially distinguished from bulk-soil truffles. The combined use of HS-GC–MS, GC-O, and sensory evaluation also suggests that some differences in truffle aroma could exist among natural soils. The study shows that nests are a useful tool for advancing the study of truffle ecology and indicates that there could be a possibility of managing truffle aroma with agronomic practices. Future work could aim to find physiological reasons for the loss of complexity of VOC profiles in nests, to improve techniques to correct this issue, or to check whether these observations are maintained in different climatic conditions.

ACKNOWLEDGEMENTS

The authors thank the Diputación Provincial de Huesca for the participation of the researcher Eva Gómez-Molina through the collaboration agreement for the operation of the Centro de Investigación y Experimentación en Truficultura (CIET). They also thank the Diputación Provincial de Zaragoza (project 'Mejora de la Eficiencia de la Producción y la Innovación Agroalimentaria de la Trufa en la Provincia de Zaragoza, ejercicio 2024'), and the European Agricultural Fund for Rural Development (EAFRD) 2014-2022 (project 'Organización, valorización y promoción de la Trufa Negra de Aragón').

FUNDING INFORMATION

This research was funded by the Spanish Ministry of Science, Innovation and Universities through the grant for project PID2022-139407OR-I00 'Unraveling the role of environmental factors in black truffle quality: advancing towards the management of truffle quality in the field (BOUQUET)'.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

REFERENCES

1 Campo E, Marco P, Oria R, Blanco D and Venturini ME, What is the best method for preserving the genuine black truffle (*Tuber melanosporum*) aroma? An olfactometric and sensory approach. *LWT* **80**: 84–91 (2017).
2 Trappe JM, A.B. Frank and mycorrhizae: The challenge to evolutionary and ecologic theory. *Mycorrhiza* **15**:277–281 (2005).
3 Reyna S and Garcia-Barreda S, Black truffle cultivation: a global reality. *For Syst* **23**:317–328 (2014).
4 Murat C, Bonneau L, De la Varga H, Olivier J, Sandrine F, Le TF *et al.*, Trapping truffle production in holes: a promising technique for improving production and unravelling truffle life cycle. *Ital J Mycol* **45**:57–53 (2016).
5 García-Barreda S, Marco P, Martín-Santafé M, Tejedor-Calvo E and Sánchez S, Edaphic and temporal patterns of *Tuber melanosporum* fruitbody traits and effect of localised peat-based amendment. *Sci Rep* **10**:1–9 (2020).
6 UNECE, *Standard FFV-53 Concerning the Marketing and Commercial Quality Control of Truffles*. United Nations, Geneva (2023).
7 Caboni P, Scano P, Sanchez S, Garcia-barreda S, Corrias F and Marco P, Multi-platform metabolomic approach to discriminate ripening markers of black truffles (*Tuber melanosporum*). *Food Chem* **319**: 126573 (2020).
8 Culleré L, Ferreira V, Marco P, Venturini ME and Blanco D, Does the host tree exert any influence on the aromatic composition of the black truffle (*Tuber melanosporum*)? *Flavour Fragr J* **32**:133–140 (2016).
9 Splivallo R, Deveau A, Valdez N, Kirchhoff N, Frey-Klett P and Karlovsky P, Bacteria associated with truffle-fruiting bodies contribute to truffle aroma. *Environ Microbiol* **17**:2647–2660 (2015).
10 Culleré L, Ferreira V, Chevret B, Venturini ME, Sánchez-Gimeno AC and Blanco D, Characterisation of aroma active compounds in black truffles (*Tuber melanosporum*) and summer truffles (*Tuber aestivum*) by gas chromatography-olfactometry. *Food Chem* **122**: 300–306 (2010).
11 Tejedor-Calvo E, García-Barreda S, Sánchez S, Morales D, Soler-Rivas C, Ruiz-Rodriguez A *et al.*, Supercritical CO_2 extraction method of aromatic compounds from truffles. *LWT* **150**:111954 (2021).
12 Martin F, Kohler A, Murat C, Balestrini R, Coutinho PM, Jaillon O *et al.*, Périgord black truffle genome uncovers evolutionary origins and mechanisms of symbiosis. *Nature* **464**:1033–1038 (2010).
13 Zeppa S, Guidi C, Zambonelli A, Potenza L, Vallorani L, Pierleoni R *et al.*, Identification of putative genes involved in the development of *Tuber borchii* fruit body by mRNA differential display in agarose gel. *Curr Genet* **42**:161–168 (2002).
14 Dravnieks A, *Atlas of Odor Character Profiles*. ASTM, Philadelphia, PA (1985).
15 ISO 4120:2021, Sensory analysis. Methodology Triangle test European Committee for Standardization (2021).
16 Rivera CS, Venturini ME, Oria R and Blanco D, Selection of a decontamination treatment for fresh *Tuber aestivum* and *Tuber melanosporum* truffles packaged in modified atmospheres. *Food Control* **22**:626–632 (2011).
17 Culleré L, Ferreira V, Venturini ME, Marco P and Blanco D, Chemical and sensory effects of the freezing process on the aroma profile of black truffles (*Tuber melanosporum*). *Food Chem* **136**:518–525 (2013).
18 Abu-Hamdeh NH and Reeder RC, Soil thermal conductivity effects of density, moisture, salt concentration, and organic matter. *Soil Sci Soc Am J* **64**:1285–1290 (2000).
19 Michel JC, The physical properties of peat: a key factor for modern growing media. *Mires Peat* **6**:1–6 (2010).
20 Argo WR, Root medium chemical properties. *Horttechnology* **8**:486–494 (1998).
21 Garcia-Barreda S, Marco P, Bonito G, Parlade J, Sánchez S, González V *et al.*, Interannual Dynamics of Tuber melanosporum and Fungal Communities in Productive Black Truffle Orchards Amended with Truffle Nests. *FEMS Microbiol Ecol* **99**:1–12 (2023).
22 Molinier V, Murat C, Frochot H, Wipf D and Splivallo R, Fine-scale spatial genetic structure analysis of the black truffle *Tuber aestivum* and its link to aroma variability. *Environ Microbiol* **17**:3039–3050 (2015).

Journal of The Science of Food and Agriculture published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry.