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Spatial distribution and ecological variation of re-discovered German truffle habitats

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ABSTRACT

Several truffle species (*Tuber* spp.) are highly prized by chefs and gourmets with some commanding prices of up to €9.000 kg⁻¹ on international markets. Their ecological drivers and geographical patterns, however, often remain a puzzle. Truffle species in Germany are classified as Very Rare or even Extinct on the national Red Lists, while historical literature described their sporadic existence. Here we present evidence of seven *Tuber* species (*T. aestivum*, *T. brumale*, *T. excavatum*, *T. fulgens*, *T. macrosporum*, *T. mesentericum*, *T. rufum*), discovered at 121 sites in Southwest Germany. The valuable Burgundy truffle (*T. aestivum*) occurred at 116 sites. An unexpected abundance of *Tuber* spp. associated with 13 potential host plants along wide ecological gradients in a region far outside the traditional Mediterranean truffle foci in France, Italy and Spain, is likely indicative of possible responses to climate change, and also suggests ample truffle cultivation potential north of the Alpine arc.

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Introduction

Truffles are hypogeous fruit bodies of ascomycete fungi belonging to the genus *Tuber* (Trappe 1979). To date, it is widely accepted that *Tuber* spp. occur exclusively in association with certain host plants and only under specific ecological habitat characteristics, including a narrow range of climatic conditions (Hall *et al.* 2007). The worldwide number of *Tuber* spp. remains unclear, but it is assumed to exceed 180 species (Bonito *et al.* 2010). Some of these have outstanding commercial value and command prices up to €9.000 kg⁻¹ (Hall *et al.*

2007), whereas many species do not have culinary value to humans (Pacioni *et al.* 1990). For spore dispersal, an array of wildlife animals, including boar, mice and squirrel, is attracted by the aromatic compounds and consumes the fruit bodies after digging them out. The spores then pass unharmed through the digestion system and are dispersed with the dung in potentially new habitats (Trappe & Claridge 2010). Although many physiological parts of the distribution strategy of *Tuber* spp. are understood, the overall bio-geographical and site-ecological requirements of many species still remain unclear (Büntgen *et al.* 2011). Aside from the traditional truffle

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cultivation hotspots in France (Midi-Pyrenees and Provence for *Tuber melanosporum* and Burgundy for *Tuber aestivum*), Northern and Central-Italy (both species) and Central Spain for *T. melanosporum*, newly discovered truffle grounds like the Swedish island of Gotland, have gained in importance with a concomitant impact on its rural economy (Weden et al. 2009). Detailed insight on the Burgundy truffle (*T. aestivum* syn. *uncinatum*; Weden et al. 2005), which is assumed to be most widespread across Europe, is therefore not only of particular scientific interest but also of enormous economic value (Hall et al. 2003).

A decline in truffle harvest across the Mediterranean area, possibly related to increasing temperature means and decreasing precipitation totals (Büntgen et al. 2011), and a decreasing supply with rising market prices has subsequently led to an upsurge in interest in truffle cultivation (Martin 2011). An expected humidity loss in the Mediterranean basin, especially in summer (Gibelin & Deque 2003; Thuiller 2007), is likely to further add to this issue. In contrast, habitats suitable for fungal growth in more northern parts of the temperate European forest zone are likely to increase due to a slight temperature increase (Büntgen et al. 2011), associated with extended vegetation periods and less frequent and severe frost events (Menzel & Fabian 1999; Menzel et al. 2003). Indeed, various species-specific geographical range shifts, mostly towards higher elevations and northern latitudes, have been reported (Burrows et al. 2011) and may also account for a spatial expansion of truffle habitats in tandem with improved growth conditions of their host plants (Büntgen et al. 2011).

Historical German literature described the occurrence of different *Tuber* spp., as well as the utilisation of *T. aestivum* mainly for manufacturing sausage (*Leberwurst*; Hesse 1891). Hesse (1891) also provided information on the harvesting of *T. aestivum* throughout Germany and described several new species including *Tuber debaryanum*, *Tuber exiguum*, *Tuber murinum*, *Tuber rutilum* and *Tuber scruposum*. To our knowledge, very little information about the ecology and distribution of *Tuber* spp. has been published since then, and many species have even been regarded as extinct, or near so (Ludwig & Schnittler 1996). The Red List of German Mushrooms (Ludwig & Schnittler 1996), the Red List of Baden-Württemberg Mushrooms (Winterhoff & Krieglsteiner 1984), and an atlas of fungal distribution (Krieglsteiner 1993) are currently the only sources that contain information on truffle distribution in Germany. Other publications partly describe diversity, geography and ecology, but do not provide a sufficient overview (Pacioni 1981; Breitenbach & Kränzlin 1984; Gerhardt 1996). In contrast, ample truffle findings have been reported from countries nearby Germany, such as France (Chevalier & Frochot 1997a), Switzerland (Swiss Federal Institute for Forest, Snow and Landscape Research WSL 2010), Austria (Urban et al. 2004), Denmark (Global Biodiversity Information Facility GBIF 2009) and Poland (Hilszczańska et al. 2008; Lawrynowicz et al. 2008).

Here we explore the distribution of *Tuber* spp. in Southwest Germany and seek to understand their geographical and ecological requirements using morphological and molecular methods. Results are discussed in light of on-going climate change and concluding remarks focus on possible cultivation endeavours.

Methods

The research area in Southwest Germany (Baden-Württemberg) is adjacent to well-known truffle habitats in France (Burgundy and Lorraine) and Switzerland (Jura). Additionally, the climate is similar to the mentioned regions and there is an abundance of calcareous geological formations, essential for the growth of almost all *Tuber* spp. (Sourzat 2002; Weden et al. 2004; Hall et al. 2007). Search areas were defined on the basis of geology and site characteristics in the southern Rhine valley, the Hegau region and the southern Swabian Jura, whereas the acidic areas of the Black Forest were excluded. Potential sites were identified using presence of suitable trees or shrubs, biological structures like forest edges, topographical features like relief and exposure, while unlikely areas such as agricultural land were excluded. Well-trained truffle dogs of the breeds *Bracco francese* and *Lagotto romagnolo* were used to locate truffles between 2008 and 2011. The dogs are able to detect the odour of many hypogeous fungi and do not discriminate between different *Tuber* species or other species such as *Choiromyces meandriformis* and *Melanogaster* spp. The dogs often explored areas, which were unlikely to be truffle habitats, and surprisingly, yielded additional findings. Most sites were frequently visited throughout the 3-yr inventory to assess truffle occurrence in different seasons, and thus gain insight into their intra-annual phenology.

Wherever truffle fruit bodies were found, geographical coordinates were determined by GPS, and all tree and shrub species were recorded to describe the characteristic floristic composition of the truffle site. The relative proportion of tree species at 71 *T. aestivum* sites was also systematically observed to assess its likely hosts. Geological parent material was defined by the LGRB map-server (Regierungspräsidium Freiburg 2011). Temperature and precipitation data from 73 meteorological stations within the research area (~2 500 km² with a 10 km buffer zone) was obtained from the DWD (Deutscher Wetterdienst; Federal Ministry of Transport, Building and Urban Development). Site locations were illustrated on 10 × 10 km gridded high-resolution maps allowing evaluation against the Red Lists (Winterhoff & Krieglsteiner 1984; Ludwig & Schnittler 1996).

The identity of each fruit body was determined from macroscopic and microscopic features following Pacioni (1981), Breitenbach & Kränzlin (1984), Sourzat (2002), the internet platform Tuberkey (University of Parma 2006), Hall et al. (2007), and the key provided by Trappe & Castellano (2007). Microphotographs were prepared using a ZEISS Axio-phot light microscope at 50–400× magnification fitted with a ZEISS AxioCam MRc 5 digital camera. The shape (net structure or spines on the surface) and size (length × width in μm) of the spores were the most important features.

The internal transcribed spacer regions (ITS1 and ITS2) of the truffle DNA were analysed with molecular genetic methods when the identification by macroscopic and microscopic methods left doubts. At least three samples of each species were studied and the best sequence was used for identification (GenBank accession nos. JF926117, JF926118, JF926119, JF926120, JF926121, JF926122, JF926123). DNA extraction was performed with the DNeasy Plant Mini Kit

(Qiagen) following the company's protocol, using freeze-dried sample material. DNA-concentrations of the extracts were measured with a NANOPhotometer (Implen) and the extracts were then adjusted to a concentration of $\sim 20 \mu\text{g DNA l}^{-1}$. For the PCR reaction, a Mastercycler ep (Eppendorf) was used with the primers ITS5/ITS4 for *T. aestivum*, *Tuber mesentericum*, *Tuber brumale*, *Tuber fulgens*, *Tuber rufum* and for *Tuber excavatum* in addition ITS1F/ITS4A. For *Tuber macrosporum*, ITS5/ITS4A and ITS1/ITS4A were used. The different primer pairs resulted in a varying quality of PCR results. Species identification through sequence analysis could therefore be enhanced. The PCR of all samples was carried out with the programs described by White *et al.* (1990). Only the second sequencing of the same amplification product using the backward primer resulted in a complete sequence of *T. aestivum*. A usable sequence of the *T. macrosporum* ITS region could be obtained by combining two fragmented sequences from different amplifications with the primer pairs ITS1/ITS4A and ITS5/ITS4A. Amplification products were separated by agarose gel electrophoresis and were visualized by ethidium bromide under UV-light. They were cleaned with a PCR-cleaning kit (Seqlab) and their concentration was measured with a NANOPhotometer (Implen). Sequencing was accomplished by Seqlab (Sequence Laboratories, Göttingen). Sequence analysis was performed by using the program Chromas 2.33 (Technelysium Pty Ltd). BLAST searches in GenBank (NCBI) were conducted to determine the different *Tuber* spp.

Results

Site ecology

Seven different *Tuber* species were found at 121 sites (Fig 1) on more than 150 excursions throughout the 3-yr monitoring period from 2008 to 2011 (Table 1). *T. aestivum* was most frequently found at 116 sites. The most commonly recorded trees on these sites were *Quercus robur* (69 sites), *Fagus sylvatica* (68 sites), *Acer campestre*, (22 sites) and *Carpinus betulus* (17 sites). Particularly *A. campestre* as an accompanying non-host tree, was a regular part of the floristic composition on *T. aestivum* sites. The most common shrubs were *Cornus* spp. (57 sites), *Prunus spinosa* (46 sites), *Corylus avellana* as the only shrub host (45 sites), *Crataegus* spp. (39 sites) and *Ligustrum vulgare* (32 sites). *T. aestivum*, however, also occurred beneath conifers such as *Picea abies* (15 sites). Most unexpected vegetation associations were *P. abies* with *Cornus* spp. and *Sambucus* spp. (one site) and *Abies alba*, *Fraxinus excelsior* and *Sambucus* spp. (one site). One location was characterized by *Betula pendula* with *P. spinosa* and *Cornus* spp. *Quercus petraea* was recorded only at three out of the 116 sites and thus plays a minor role as a symbiotic partner of *Tuber* spp. in the research area. Fig 2 illustrates the relative proportion of all trees and shrubs on *T. aestivum* sites with potential host plants highlighted.

A broad range of calcareous bedrocks was associated with the truffle sites. The Jurassic rock formations of the Swabian Jura and the Rhine valley slopes, as well as Quaternary glacial deposits of the northern pre-Alps, were

the most common bedrocks, followed by freshwater molasses, loess and volcanic tuff. The climatic envelope for *T. aestivum* ranges at least from 700 to 1 200 mm annual precipitation and has a mean annual temperature range of 7.1–10.5 °C. Site elevation varies from 212 to 950 m with *T. aestivum* representing the full gradient. The mean annual precipitation in the research area is 825 mm with generally more rainfall between May and Aug.. Mean temperatures range from -0.7 °C in Jan. to 17.4 °C in Jul. with an annual mean of 8.25 °C.

T. excavatum occurred at 21 sites (always coexisting with *T. aestivum*) with similar host vegetation, as well as geological and climatic conditions. *T. excavatum* was not present towards the western margin of the research area. *T. brumale* (five sites), *T. mesentericum* (seven sites) and *T. macrosporum* (two sites) were verified at sites with a maximum elevation of 483 m and a mean annual temperature above 9 °C. *T. brumale* was exclusively associated with *F. sylvatica* and was never found beneath *Q. robur*. At the most productive location on the Rhine valley slope, the vegetation included *F. sylvatica* and *A. alba*. This particular site (Fig 3B) yielded more than 300 fruit bodies during Feb. and Mar. 2011 despite regular collecting every 2 weeks. *T. macrosporum* was exclusively associated with *C. avellana* on Quaternary glacial deposit. *T. rufum* (eight sites) and *T. fulgens* (three sites) occurred at very similar settings to those of *T. excavatum* and *T. aestivum*. Their main hosts are *Q. robur* and *F. sylvatica* in combination with *C. avellana*, but without *C. betulus*. *T. fulgens* was generally collected at higher elevations (639–784 m) than *T. aestivum* (212–950 m), while *T. rufum* was found at approximately similar elevations (472–696 m). Site characteristics are summarized in Table 1. Site elevations sorted by species are shown in Fig 4.

Taxonomic identification

Macroscopic identification of *T. aestivum* was always successful. Some samples of *T. excavatum* and *T. fulgens* (yellow colour and warts with <1 mm diameter) were difficult to distinguish because of the inconsistent presence of discriminatory characteristics. *T. mesentericum* frequently lacked a distinctive cavity, but, nevertheless, could easily be separated from the similar *T. aestivum* by its strong tar-like smell. *T. aestivum* formed particularly large fruit bodies up to 414 g (Büntgen *et al.* 2011, Fig 3D) and had a mean weight of 21.2 g among 322 fruit bodies. *T. brumale* reached a maximum weight of 57 g and had a mean weight of 2.69 g among 127 fruit bodies. *T. macrosporum* took an intermediate position with approximately 30 g per specimen. Spore examinations resulted in a species identification due to the spore length of up to 68 μm , compared to a maximum of 36 μm of the morphologically similar *T. aestivum* spores. Distinctive spines, significantly different from the net structure of every other investigated species were observed on *T. rufum* and *T. brumale* spores. However, the identification by spore examination was insufficient, because the spore size and morphology of the two species could not be distinguished. Generally, a combination of macroscopic and microscopic features yielded exact results in these cases. In addition, all species were successfully identified using the molecular techniques.

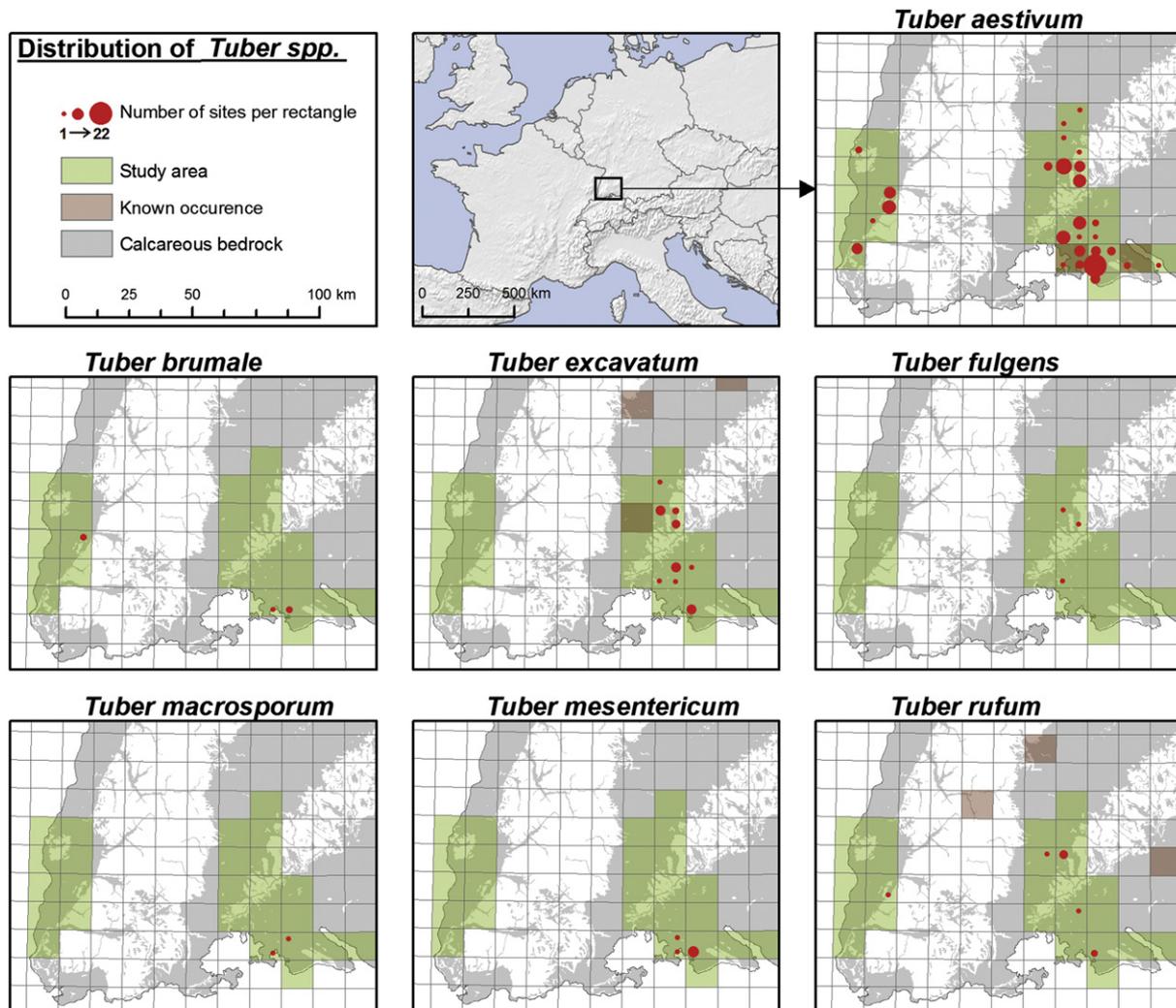


Fig 1 – Distribution of *Tuber* spp. in Baden-Württemberg. The grid equals the partition of the area in 1:25 000 ordnance survey maps and was used for mapping in previous Red Lists. Areas with potential calcareous bedrock are shown in grey. Some geological materials are not exclusively calcareous and are therefore not indicated. Every red dot marks the occurrence of a *Tuber* sp. within a quarter of the ordnance map. The sites were quantified by the size of the dots. Searched areas are green and ordnance maps with previously reported occurrences (Krieglsteiner 1993; Ludwig & Schnittler 1996) are coloured brown.

Discussion

Site ecology

Due to the indispensable assistance of trained truffle dogs, a total of 121 truffle sites were discovered between 2008 and 2011 in Southwest Germany. *T. aestivum* was found at 116 sites. This surprising result contrasts with the common belief that the species is endangered (Ludwig & Schnittler 1996). *T. aestivum* was discovered in large quantities along broad ecological gradients, including numerous different geological bedrocks (Jurassic rocks, glacial deposits, molasses, loess, calcareous scree and tuff), and a great variety of associated host plants, of which *Q. robur*, *F. sylvatica*, *C. avellana* were most common, and *A. campestre*, *Cornus* spp., *P. spinosa*,

Crataegus spp. and *L. vulgare* were co-occurring and contributed to a characteristic *T. aestivum* site. A similar vegetation composition occurs at truffle sites in the Swedish island of Gotland (Weden et al. 2004), although it has a dryer and cooler climate. The mean annual precipitation (1971–2000) on Gotland is 528 mm compared to 825 mm in the research area studied here and 885 mm in the traditional truffle area of Cruzy (Burgundy, France). The mean annual temperature is 6.8 °C on Gotland, 8.25 °C in the current research area, and 10.4 °C in Cruzy. Hall et al. (2007) provide climatic data of 29 *T. aestivum* areas among Sweden, Denmark, UK, France, Switzerland, Croatia, Italy and Spain, with annual precipitation ranges from 514 mm in Sweden to 1 545 mm in Switzerland with an overall mean of 750 mm. The mean temperature is 3.5 °C for Jan. and 17.3 °C for Jul. compared to –0.7 °C and 17.4 °C in the current research area. With only two

Table 1 – *Tuber* species in the state of Baden-Württemberg and information about their ecology: the status in the Red List translates to 0 = disappeared/extinct, 1 = endangered of extinction, 2 = critically endangered, 3 = endangered, R = rare but not endangered (Ludwig & Schnittler 1996). *Tuber fulgens* and *T. rufum* are not yet listed but found in our investigations. *Tuber macrosporum* as well as *T. mesentericum* were considered extinct. *Tuber aestivum*, together with *T. excavatum* and *T. rufum* appeared to have the widest range of tree and shrub species at its sites, the diversity of geological parent material and the broad range of elevation, mean ann. precipitation and mean ann. temperature. *Tuber mesentericum* and *T. brumale* seem to prefer lower elevations and a warmer and dryer climate. *Tuber fulgens* takes an intermediate position

	<i>Tuber aestivum</i>	<i>Tuber excavatum</i>	<i>Tuber fulgens</i>	<i>Tuber mesentericum</i>	<i>Tuber brumale</i>	<i>Tuber macrosporum</i>	<i>Tuber rufum</i>
Status in Red List	1	R	Not listed	0	1	0	Not listed
Number of sites	116	21	3	7	5	2	8
Trees on sites (potential host plants bold)	quro, qupe, fasy, cabe, acca, tisp, acps, frex, piab, abal, casa, pisy, ulsp, posp, bepe	quro, fasy, acca, tisp, acps, frex, piab, abal, prsp, pisy	quro, fasy, acps, frex, piab, prsp, pisy	quro, fasy, acca, frex, prsp, pisy, bepe, rops	fasy, abal, prsp	No trees on sites	quro, fasy, acca, acps, frex, piab, prsp, pisy
Shrubs on sites (potential host plants bold)	coav, crsp, prsa, cosp, livu, rosp, ilaq, sasp, eueu, soau, vibla, rusp	coav, crsp, prsa, cosp, livu, rosp, sasp, soau	coav, crsp, prsa, cosp, livu, sasp	coav, crsp, livu, sasp	coav, crsp, livu	coav, crsp, livu	coav, crsp, prsa, cosp, livu, rosp, sasp
Geological parent material	Jurassic rocks, glacial deposits, molasses (conglomerate), loess, calcareous scree, tuff	Jurassic rocks, glacial deposits	Jurassic rocks	Glacial deposits, molasses (conglomerate)	Jurassic rocks, glacial deposits	Glacial deposits	Jurassic rocks, glacial deposits
AMSL (m) (min/max, mean)	212/950 (541)	413/806 (627)	639/784 (701)	410/483 (435)	393/480 (433)	438, 440	472/696 (595)
Ann. prec./mm (min/max, mean)	750/1 200 (880)	750/950 (844)	800–900	800/900 (832)	300/1 100 (915)	800–850	800/1 100 (878)
Ann. temp./°C (min/max, mean)	7.1/10.5 (8.67)	7.1/9.5 (8.16)	7.6–8.0	8.1/9.5 (9.09)	8.6/10.0 (9.4)	8.6–9.5	7.6/10.0 (8.43)
Months of occurrence	Jan.–Dec.	Jun.–Nov.	Jun.–Nov.	Jul.–Nov.	Nov.–Mar.	Oct.–Nov.	Jun.–Nov.
<p><i>Quercus robur</i> = quro, <i>Quercus petraea</i> = qupe, <i>Fagus sylvatica</i> = fasy, <i>Carpinus betulus</i> = cabe, <i>Acer campestre</i> = acca, <i>Tilia</i> spp. = tisp, <i>Acer pseudoplatanus</i> = acps, <i>Fraxinus excelsior</i> = frex, <i>Picea abies</i> = piab, <i>Abies alba</i> = abal, <i>Castanea sativa</i> = casa, <i>Pinus sylvestris</i> = pisy, <i>Ulmus</i> spp. = ulsp, <i>Populus</i> spp. = posp, <i>Betula pendula</i> = bepe, <i>Robinia pseudoacacia</i> = rops, <i>Prunus</i> spp. = prsp, <i>Corylus avellana</i> = coav, <i>Crataegus</i> spp. = crsp, <i>Prunus spinosa</i> = prsa, <i>Cornus</i> spp. = cosp, <i>Ligustrum vulgare</i> = livu, <i>Rosa</i> spp. = rosp, <i>Ilex aquifolium</i> = ilaq, <i>Sambucus</i> spp. = sasp, <i>Euonymus europaeus</i> = eueu, <i>Sorbus aucuparia</i> = soau, <i>Viburnum lantana</i> = vibla, <i>Rubus</i> spp. = rusp.</p>							

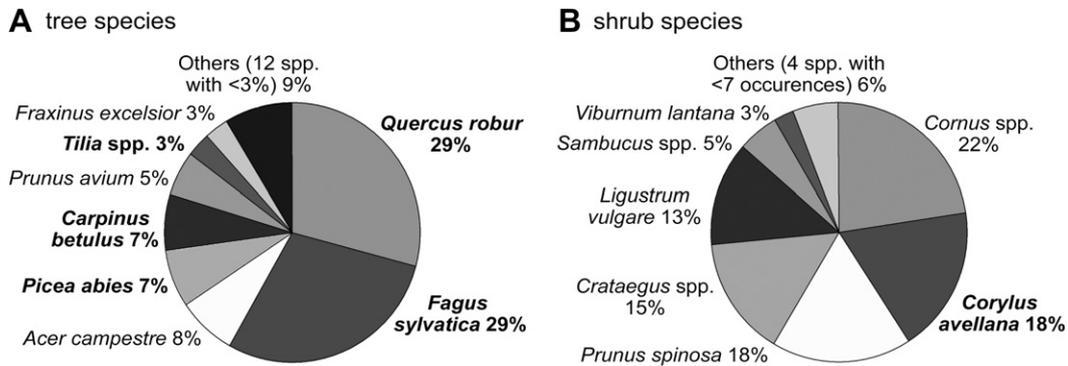


Fig 2 – (A) Abundance of tree species at 71 *T. aestivum* sites recorded by percentage of their occurrence at each site. (B) Abundance of shrub species at all 116 *T. aestivum* sites recorded by presence or absence. Potential host plants are highlighted with bold text.

regions in this dataset climatically comparable to Germany, more continental regions with cooler winters are underrepresented, although *T. aestivum* is widely distributed there. An overview of European truffle regions and proven find locations within the research area at the geographical centre of the distribution range are shown in Fig 3A. We conclude that *T. aestivum* in Southwest Germany is currently growing at, or at least near, its ecological optimum. The great abundance of the species in the research area and the growth of single fruit bodies up to the exceptional size of 414 g support this assumption (Büntgen et al. 2011).

The species *T. excavatum* (21 sites) and *T. rufum* (eight sites) also appear to be well adapted to the ecological conditions of the research area, but are less frequent compared to *T. aestivum*. *T. excavatum* is classified as Rare but not endangered on the Red List, which we confirm. *T. rufum* is not listed at all but

should achieve the same status. *T. mesentericum* and *T. macrosporum* are classified as Extinct and, along with *T. brumale* (classified as endangered), occur in the research area at low elevations and mean temperatures above 9 °C. The three species are well known from Mediterranean regions (Hall et al. 2007), and appear to be very rare in our research area. Sourzat (2008) described an affinity of *T. brumale* to *C. avellana* and *Quercus* spp. as host trees in France. In contrast, *F. sylvatica*, the most common host tree in the present study was not mentioned. The vegetation at two sites consisted of *F. sylvatica*, with *Prunus* spp. and *Cornus* spp. as co-occurring vegetation, but actually lacked the presence of *Quercus* spp. or *C. avellana* as possible host trees.

Despite extensive surveys throughout the years no *Tuber borchii* fruit bodies were found. The species is classified as endangered in Germany (Ludwig & Schnittler 1996), but is

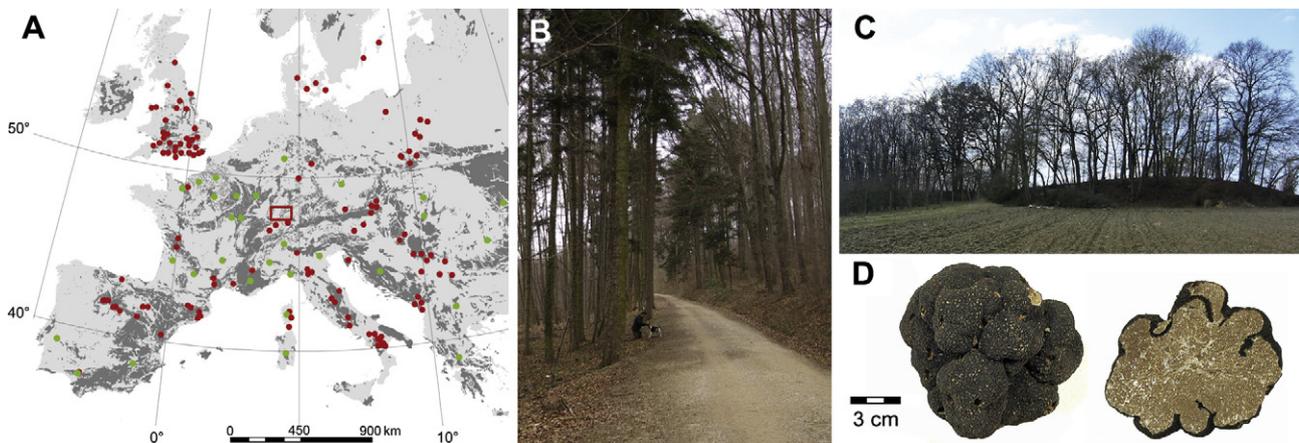


Fig 3 – (A) Europe with regions of calcareous geology (potential truffle habitats) in grey. Exact find locations of *T. aestivum* as well as the research area (square) are indicated in red. Areas (countries/states) with *T. aestivum* occurrences indicated in green. Some geological materials like glacial deposits are not exclusively calcareous and are therefore not indicated. Base map: European Communities (1995–2010) – European soil portal. Find locations: Lubelska (1953), Chevalier (1978 & pers. comm. 2011), Lawrynowicz (1992), Hall et al. (2007), Pomarico et al. (2007), Global Biodiversity Information Facility GBIF (2009), Lawrynowicz et al. (2008), Marjanovic et al. (2009), Weden et al. (2009), Swiss Federal Institute for Forest, Snow and Landscape Research WSL (2010). (B) Exceptional *T. brumale* site on the Rhine valley slope characterized by *Abies alba* and *Fagus sylvatica* as host trees. (C) Typical *T. aestivum* and *T. mesentericum* site in the Hegau region. (D) Fruit body of *T. aestivum* with the exceptional size of 414 g found in the Hegau region with a brown, mature gleba (Büntgen et al. 2011).

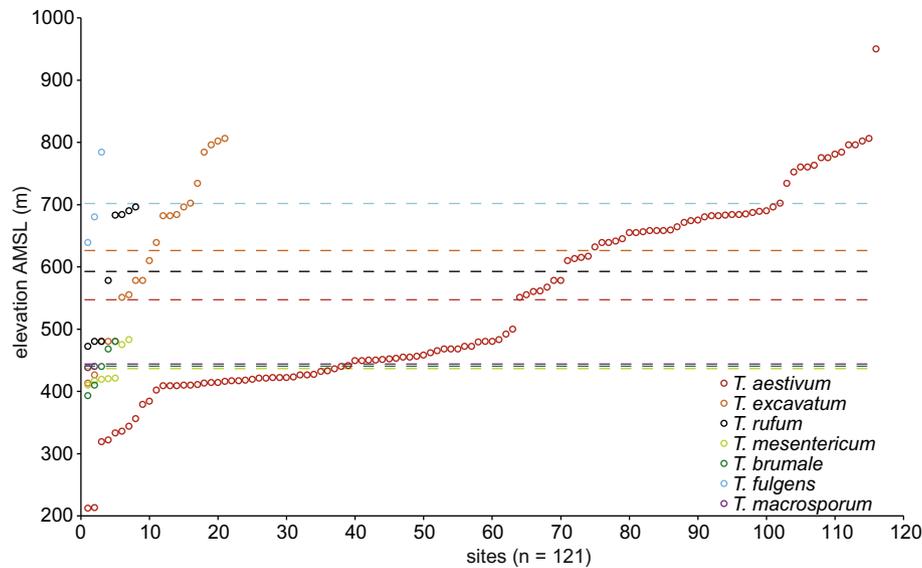


Fig 4 – Elevation of *Tuber* spp. sites in m AMSL sorted by species. The broken lines illustrate the mean elevation of each species.

described as having a wide distribution throughout Europe (Hall *et al.* 2007). Reasons for this result may be misidentifications in the past or simply a low abundance in the research area. *T. scruposum* (endangered) was described by Hesse (1891) and on the Red List (Ludwig & Schnittler 1996), *Tuber rapaeodorum* (endangered) and *Tuber puberulum* (critically endangered) were described for the area by Krieglsteiner (1993), but, were not found during our survey.

Climate change

Truffle findings outside the Mediterranean region and the well-known truffle areas may be early indicators for ecological responses to climatic changes, or simply emphasize our incomplete knowledge of their natural distribution. A frequently addressed phenomenon is the shift of geographic ranges towards the poles and higher elevations. This affects not only the species distribution boundaries, but also the core of their ranges (Lenoir *et al.* 2009). Thuiller (2007) described a shift of ecological zones in the Northern Hemisphere by ~160 km northward per 1 °C temperature change. Büntgen *et al.* (2012) pointed to an average warming trend of 0.5 °C per century with a northward shift of ~110 km. For Southwest Germany a warming by approximately 1 °C over the past century and another predicted 2 °C until 2100 in a modest scenario (Walther *et al.* 2002), would shift the recent ecological optimum of Mediterranean species like *T. brumale* north of the Alpine arc.

T. aestivum has a wide ecological and geographical range, and a southern distribution boundary in North Africa (Chevalier & Frochot 1997a; Hall *et al.* 2007). Hence, climate change would not affect *T. aestivum* as much as species with smaller distribution ranges (Pompe *et al.* 2008). More likely would be an adjustment in host tree association from *F. sylvatica* towards *Quercus* spp. within the research area, since *F. sylvatica* is assumed to suffer from increased drought (Geßler *et al.* 2007; Friedrichs *et al.* 2008, 2009). The ecology of

T. brumale in the research area, with a host tree preference to *F. sylvatica*, differs significantly from the characterisation (Sourzat 2008) provided for Southern France. It is uncertain if *T. brumale* will occur with *Quercus* spp. as host tree in the future, move northward along with *F. sylvatica*, or both possibilities. We assume that *T. brumale* and *T. macrosporum* are nearly at the northern boundary of their distribution in Southwest Germany. According to Lawrynowicz (1992), *T. brumale* is distributed up to 52°N, which is about 400 km north of our research area. Both species would therefore benefit from a changing climate towards warmer and dryer conditions.

On the basis of our survey, we assume that a limiting factor for truffle growth is soil frost. With an extended growing season and less frost events in spring and autumn (Menzel *et al.* 2003), truffle growth should extend seasonally. On the other hand, snow cover helps to prevent soil frost and enables truffles to grow longer. Our findings of *T. aestivum* and *T. brumale* under snow until Mar. confirm this. Without snow cover, the first severe soil frosts probably put a sudden end to truffle growth, even if the growing season is generally prolonged.

Understanding of how truffle fruiting is affected by the reported phenological shift of their host trees (Menzel & Fabian 1999) is ambiguous due to a lack of sufficiently long and highly resolved monitoring evidence. Kauserud *et al.* (2008, 2009) described a delayed autumnal fruiting of mushrooms in Norway by 12.8 d since 1980 and earlier spring fruiting by 18 d since 1960, coincident with an extended growing season. Since most truffle finds occurred in autumn and winter around and after leaf fall, and none after bud break, it is likely that the truffle growing season will be compressed. Information on the phenology of *Tuber* spp. in relation to their host trees is generally missing. We suggest launching systematic and regular high-resolution monitoring surveys to assess the influence of climatic variations on truffle production. In this context, one useful indicator of growth activity could be the

ring-shaped microstructures on the warts of *T. aestivum* fruit body peridium reported by Büntgen et al. (2011). They appear on all warts of a fruit body in a similar spacing and seem to be a result of possible daily growth increment.

Cultivation potential

Our results indicate great cultivation potential for Southwest Germany. Since *T. aestivum* is of high culinary and economic value and the species is widespread in the research area, it is most suitable for cultivation. The best host tree species in Germany would be *Q. robur*, *F. sylvatica* and *C. avellana*. They appeared to be the most regularly associated host trees in our survey (Fig 2), and are well-adapted native species in the area. Truffle cultivation is a land-use form with low impact on the environment and native species used (Chevalier & Frochot 1997b). We suggest that a future cultivation in Southwest Germany should, therefore, follow the given natural guidelines. However, if the climate continues to change, and if harvest continues to decline in the Mediterranean region (Hall et al. 2003; Martin 2011) even *T. melanosporum* could be of interest for future cultivation endeavours.

Anthropogenic impact

106 out of 121 truffle sites were directly influenced by recent or historic anthropogenic disturbances such as tracks, man-made activities and boundaries to agricultural land. Structural relicts from historic agri-, silvi- and viticulture were re-occupied by natural vegetation including potential truffle host plants. Truffle sites were rarely found in silviculturally managed tree stands, which are often homogeneous in tree species and age. Areas rich in structure, however, had high tree species diversity and seem to meet the habitat requirements of *Tuber* spp. We assume that one reason may be the exposure of the ground to light, which is likely to occur wherever a homogeneous landscape is disturbed. Chevalier (1978) described a similar pattern for *T. aestivum* but states that its variety *Tuber uncinatum* seems to avoid direct sunlight. *Tuber* spp. in our research area seem to be well adapted to the predominant cultural landscapes and may even benefit from anthropogenic influences and disturbances, as long as they contribute to a structured and diverse habitat.

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