Supplementary Information

Widespread contamination of soils and vegetation with Current Use Pesticide residues along altitudinal gradients in a European Alpine valley

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Appendix

Establishment of transects and sampling

Ten transects were preselected using GIS information so that they were facing each other on the respective mountain slopes (e.g., T1 located on the western slope, directly opposite from T2 on the eastern slope). In total, odd numbered transects were located on the western and southern slopes, whereas even numbered transects were located on the eastern and northern slopes. For comparison of CUP distribution by wind onto north- and south-facing slopes, transects T3-T10 were established directly facing each other on opposite valley slopes. T11, located near Mals, was an additional transect using already existing sampling sites from a survey of the local distribution of threatened butterfly and moth species [1]. Each transect consisted of four to six individual sampling points along an altitudinal gradient (Supplementary Table 1). The individual sampling points were spaced at approximately 300 metres in altitude between them but the transects differed in the altitude range they covered, depending on mountain height and accessibility. T1 in the upper north Vinschgau was the highest transect extending from 1,461 m a.s.l. up to 2,318 m a.s.l., while sampling on T9 in the lower Vinschgau started at 517 m a.s.l. and reached a maximum altitude of 1,765 m a.s.l. (see Supplementary Table 1for individual altitudes). Further differences between transects were the dominating type of vegetation on the two mountain slopes in the valley. On the south- and west-facing transects T2, T4, T6, T8, T10 & T11, scots pine and larch forests in lower altitudes and steppe-like dry vegetation in montane altitudes dominated the landscape. In contrast, the north- and east-facing transects T1, T3, T5, T7 & T9 were characterized by dense spruce forests. Pictures of some transects with individual sampling points are provided in Appendix B. Sampling points are labelled as Tx_y whereby x indicates the number of the transect and y the individual point on the respective transect.

Sample processing & extraction

Samples were stored after sampling at 4 °C. After transport in cooling boxes to the laboratory, soil samples were frozen at -20 °C before freeze drying for 48 h (Alpha 1-4 LSC basic, Christ, Osterode, Germany). Freeze dried samples were sieved at 2 mm (Standard-compliant test sieve, ISO 3310-1, Haver

& Boecker OHG, Oelde, Germany) and stored at -20°C until extraction. For extraction, 5 g ± 0.01 g of sieved soil was weighed (ME802, Mettler Toledo, Ohio, USA, d = 0.01 g) into 50 mL tubes.
In this study, matrix-matched external calibration was used to compensate for any interferences that could affect the accuracy of the analytical method. Matrix-matched external calibration is a common alternative to internal calibration using deuterated internal standards. Matrix-matched standards were prepared using similar matrix to the actual sample being analysed. Matrix-matched standards were prepared out of soil and vegetation samples (Jockgrim, Germany, 49°05'10" N, 8°15'33" E) known to not contain measurable pesticide concentrations (blank soil and vegetation samples). For quality control, we spiked each filed soil and vegetation sample with the deuterated standard imidacloprid-D4 (98.9 %, Dr. Ehrenstorfer GmbH, Augsburg, Germany) a representative analytical standard to verify that the extraction has proceeded properly. Since deuterated internal standards are synthesized in a controlled laboratory environment and are not naturally emitted or found in the environment like pesticides or other naturally occurring substances, field samples can be spiked with them to show the reliability of the analytical method. No further deuterated standards were used for the analytical method and imidacloprid-D4 was not used to correct the measured concentrations of the CUPs. The internal standard, 50 µL of Imidacloprid-D4 in acetonitrile (MeCN, HPLC gradient grade ≥ 99.9 %, Honeywell, Charlotte, USA) was added to each sample (concentration: 10 mg L-1) and the solvent was allowed to evaporate for 30 min. The peak area of imidacloprid-D4 in field soil and vegetation samples was compared to that of the respective blank soil or vegetation sample, which was fortified with imidacloprid-D4 after extraction to check for deviations from the previously obtained extraction recovery of imidacloprid-D4.

For the salt extraction, 5 g of ammonium formate (NH_4HCO_2, reagent grade ≥ 99.0 %, Sigma-Aldrich, St. Louis, USA) and 10 mL of 2.5 % formic acid (CH_2O_2, HiPerSolv Chromanorm ≥ 99.0 %, VWR, Radnor, USA) in MeCN were added. The tubes were closed thoroughly and shaken overhead for 60 min (Stuart drive Rotator drive STR4, Cole-Parmer, Vernon Hills, USA). Afterwards, tubes were centrifuged (MegaStar 1.6R, VWR, Radnor, USA) for 6 min at 3000 rpm and the supernatant decanted through 2 µm filters (17 mm HPLC syringe filter, PTFE, hydrophobic, BGB Analytik, Lörrach, Germany) into glass tubes. The extracts were stored at -20 °C until measurement. Before analytical measurement samples were vortexed and filtered again at 2 µm into vials.

Since some soil samples (T2_4, 7_4, 8_3, 10_4) had a sponge-like consistence, probably due to high amounts of humus and dried plant fibres, an extraction with only 10 mL 2.5 % formic acid in MeCN was not possible. The extraction of the respective samples was repeated with 20 mL solvent and the dilution factor considered afterwards in the calculation when converting measured residues in mass concentrations (mass per total volume) into mass fractions (mass per total mass). A comparative extraction with 10 mL and 20 mL solvent was carried out with a test sample to ensure that the extraction volume does not lead to different results.

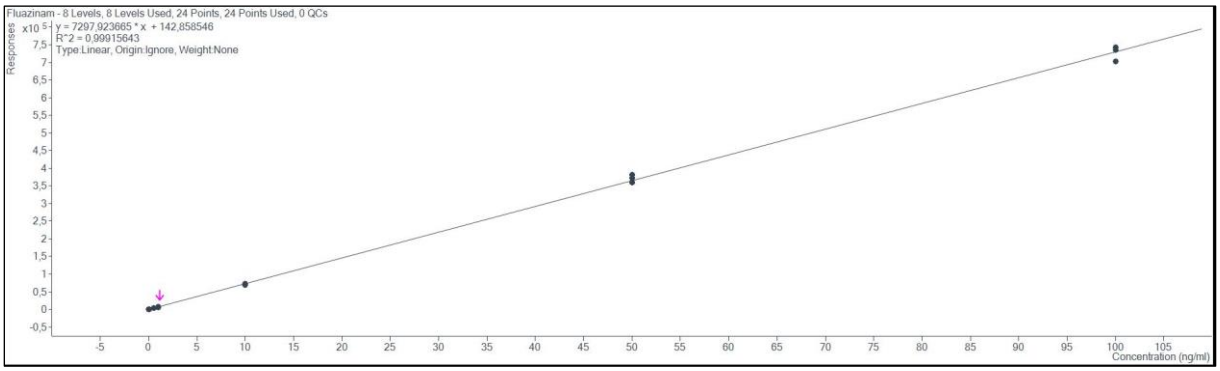
Vegetation was air-dried at room temperature for three days. The dried vegetation samples were grinded in a knife mill (GRINDOMIX GM 300, Retsch, Haan, Germany) for 2 x 1 min at 3000 rpm. After each sample, the mill was cleaned with water, soap and acetone. Grinded vegetation was stored in small freezer bags at -20°C until extraction. For extraction, 1 g ± 0.01 g of grinded vegetation was weighed (ME802, Mettler Toledo, Ohio, USA, d = 0.01 g) into 50 mL tubes. In addition, blank vegetation samples were prepared accordingly from vegetation (Jockgrim, Germany, *49°05'10" N, 8°15'33" E*) known to not contain measurable pesticide concentrations. Like soil samples, vegetation was spiked with Imidacloprid-D4 as internal standard and the solvent was allowed to evaporate for 15 min. For the salt extraction, 0.25 g of NH_4HCO_2 and 10 mL of 2.5 % CH_2O_2 in MeCN were added. Subsequent overhead shaking, centrifuging and filtering followed the methodology as described for soil samples. Before analytical measurement, chlorophyll in vegetation extracts necessitates an additional purification step by dispersive solid phase extraction (dSPE) [2]. For this, 1 mL of sample extract was added to 7.5 mg graphitized carbon black (GCB, Carbon SPE Bulk Sorbent, Agilent Technologies, Santa Clara, USA) in 2 mL Eppendorf tubes. The tubes were vortexed vigorously for 60 s and the supernatant filtered through 2 µm filters directly into the measurement vials.

CUP residue analysis

The 97 target CUPs were selected based on use records of the Julius-Kühn-Institute, Germany. CUPS frequently applied in winter wheat, oilseed rape, maize, potato and wine in 2016 and 2017. Additionally, newer insecticides such as for example chlorantraniliprole were included together with pesticides that were regularly detected in German small streams. Glyphosate and its metabolite AMPA were not included in the analytical portfolio. For quality control samples, a standard mix solution containing all 97 analytes (24 I, 37 F, 36 H) in concentrations of 5 mg L^{-1} was prepared. For stock solution preparation, single component analytical standards (Dr. Ehrenstorfer GmbH, Augsburg, Germany and Sigma Aldrich, St. Louis, USA) were separately dissolved in MeCN to 400 mg L^{-1}. The single substance stock solutions were combined with multi-component fungicide, herbicide and insecticide solutions purchased from Restek (Bellefonte, USA, concentration = 100 mg L^{-1}) as indicated in Supplementary Table 4, resulting in the final mix solution containing all analytes in concentrations of 5 mg L^{-1} in MeCN. CUP standards and working solutions were stored at -20°C.

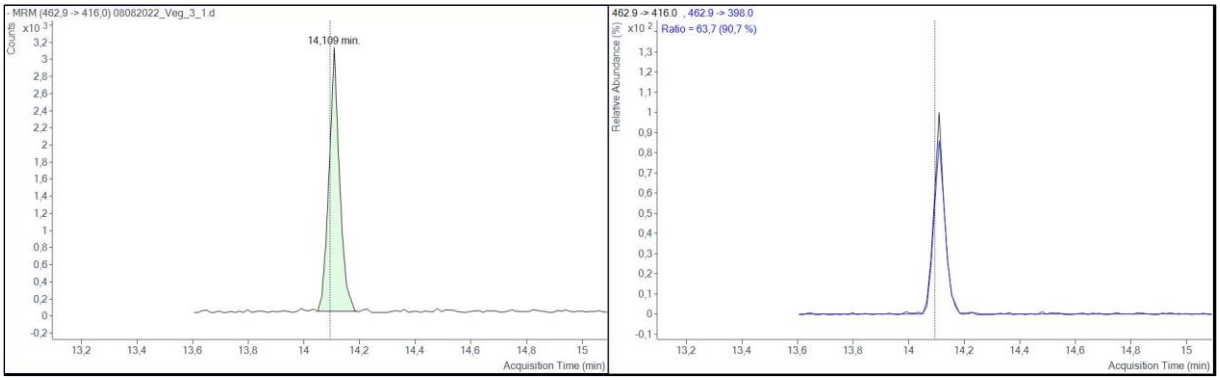
The quantification of CUP concentrations in all samples was performed using external matrix-matched calibrations. For calibration standards, blank soil, blank vegetation or blank tap water extracts, respectively, were fortified with the multi-analyte standard mix solution resulting in calibration concentrations of 0.025, 0.05, 0.1, 0.5, 1, 10, 50 and 100 µg L^{-1} (Supplementary Figure 1). The measurement of standards was repeated every 20 samples in order to account for possible system

deviations during the run time. The final calibration curve for quantification was calculated as mean of three calibration standard measurements (i.e., providing 24 calibration points at 8 concentration levels).



Supplementary Figure 1 *Matrix matched calibration curve of Fluazinam in vegetation with 8 calibration levels (0.025, 0.05, 0.1, 0.5, 1, 10, 50 and 100 µg L^{-1}).*

For the positive confirmation of an analyte following criteria were considered: The retention time of the peak had to match this of the highest concentrated calibration standard (for fluazinam here 14.109 min, Supplementary Figure 2, left) and the peak response had to lay > 1500 counts (here: 8148). The qualifier ion ratios had to lay in ranges of 70 – 130 %. The qualifier ratio here confirmed the presence of fluazinam with a qualifier ratio of 90.7 % (Supplementary Figure 2, right). Considered were also signal-to-noise ratios (S/N) of quantifier ions that had to be > 3 for a positive detection and > 10 for quantification. For quantifier ions with S/N > 3 but < 10, no quantification was possible. Pesticides detected below the determined LOD, were classified as non-detected, all others as detected. For pesticides detected at a level below LOQ but above LOD the respective LOD was used as concentration value for subsequent calculations as minimum residue that can be assumed with certainty in the sample.



Supplementary Figure 2 *Exemplary peak evaluation of Fluazinam detected in vegetation sample T3_1. Quantifier ion (left) and qualifier ion (right).*

A blank sample of MeCN as well as of the respective matrix extract was included in each measurement run to check for possible contamination. A matrix induced signal suppression or enhancement was excluded for the relevant analytes by comparison of analytical response of spiked blank samples to standards. The extraction procedure was verified with a post-spiking Imidacloprid-D4 standard that was compared to chromatographic response of pre-spiked Imidacloprid-D4 in samples.

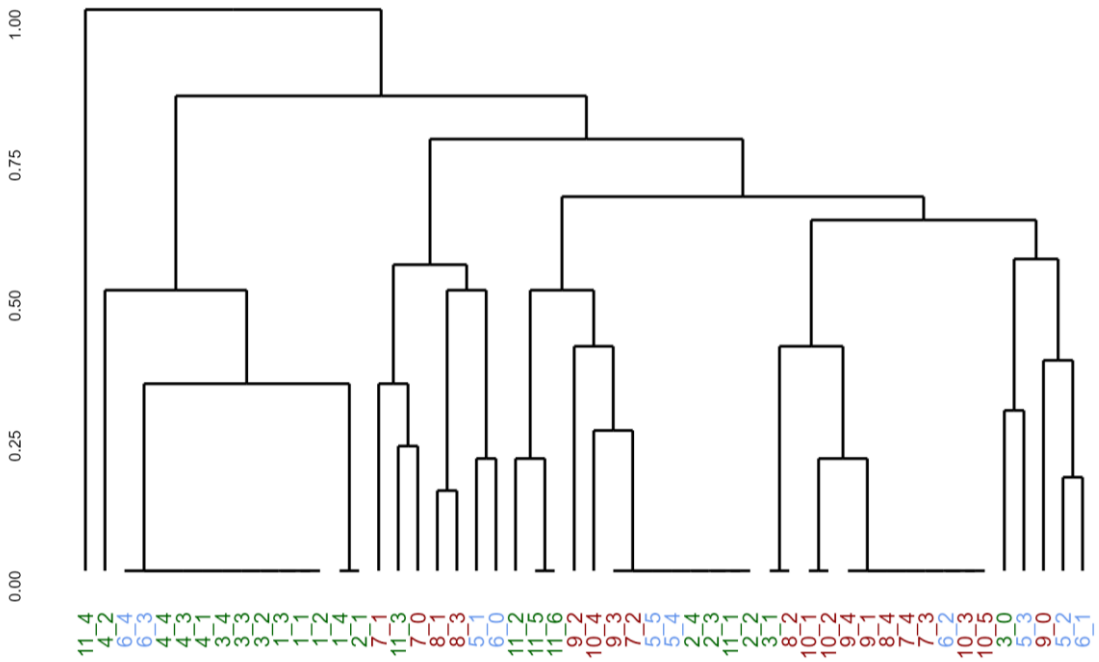
HPLC-MS/MS data was processed with Agilent Mass Hunter Quantitative Analysis software (Version 10.0., Build 10.0.707.0, Agilent Technologies, 2006 – 2018, Santa Clara CA, USA). Calibration curves showed satisfying linearity for the covered concentration range ($R^2 \geq 0.99$). Regression parameters of calibration curves were weighted when the highest calibration points dominated the calculation of the linear regression and led to an error in the lower concentration range. A weighting factor of $1/x^2$, shown to be best for bioanalytical data [3], was applied to calibration curves taking into account the high absolute variations in highest calibration points and improving the curve fit and quantification results for low concentrations [4]. Positive analyte detections were confirmed by their retention times, at least two mass transitions of qualifier and quantifier ions and the peak response.

Statistical analysis of CUP detections

Fisher's Exact Test for Count Data {stats} [5] was used to determine a potential association between the number of detected pesticides in soil and in vegetation. Shapiro-Wilk Test {stats}, [5] showed that concentration data in soil and in vegetation was not normally distributed. Levene's Test {car} [6] showed variance homogeneity for concentrations in both matrices. Due to the violation of the assumptions of normal distribution, differences in total pesticide concentrations, concentrations of pesticide groups (insecticides, fungicides, herbicides) as well as most frequently detected individual pesticides between soil and vegetation samples were assessed with Mann-Whitney U Test {stats} [5]. A paired sample correlation test after Spearman {stats} [5] was conducted to assess potential correlations between various locational factors and physical-chemical properties of CUP substances and CUP concentrations. Spearman correlation test is a non-parametric correlation test and hence can deal with the violated assumption of normal distribution in the concentration data [7]. In detail, correlations were evaluated for number of detected pesticides and measured pesticide concentrations, respectively and the altitude of the individual sampling points, their distance and altitude difference to the nearest apple orchard, the percentual area used for fruit cultivation and the pesticides' vapour pressure.

Clustering of CUPS

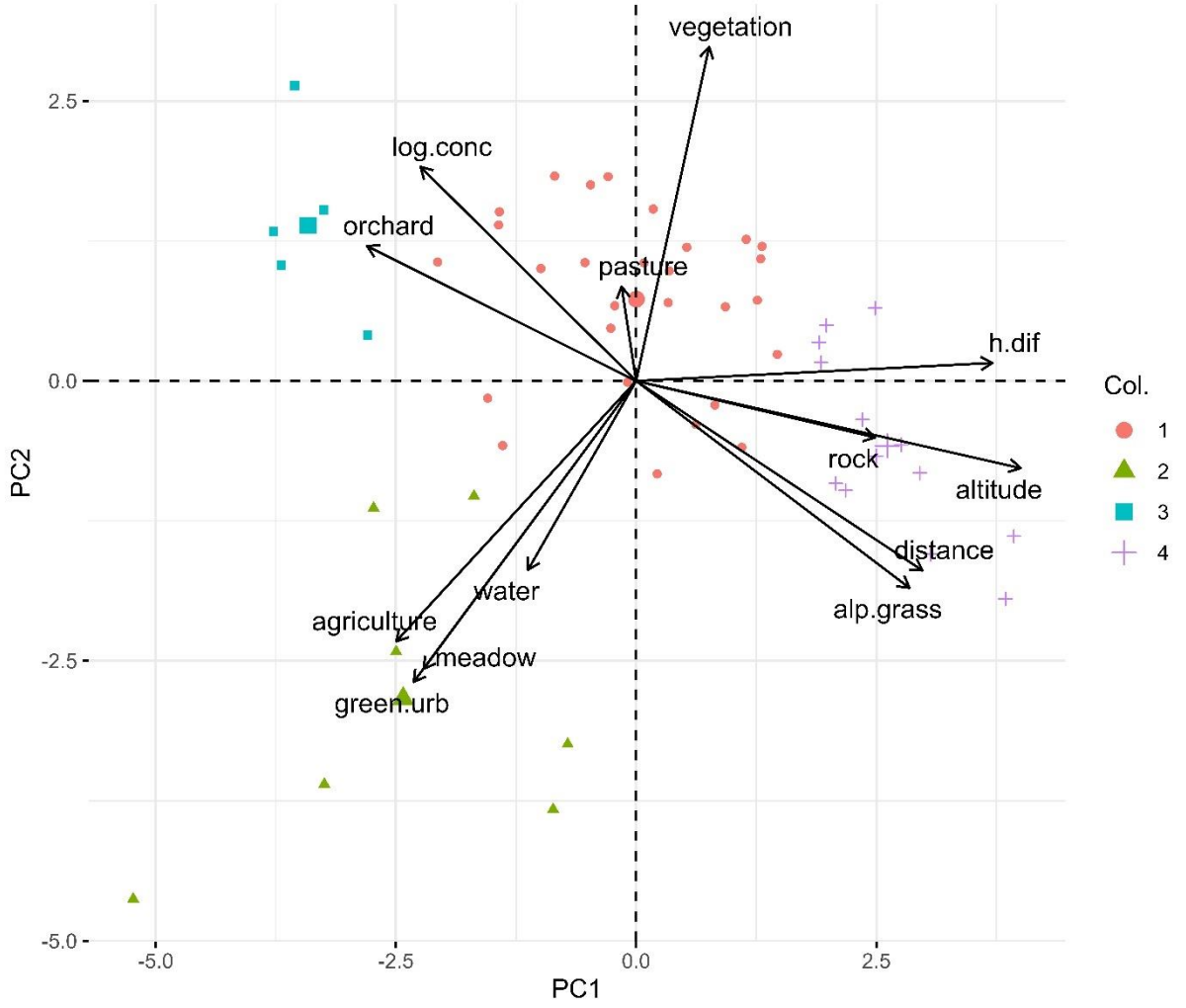
We analysed the clustering of CUPs in vegetation samples along the valley axis. As apples were already dropping flowers in the Lower Vinschgau but just at the beginning of flowering in the Upper Vinschgau it was expected that pesticide applications vary, especially insecticides are expected to be used already in the LV but not in the UV. Hierarchical clustering of pesticide detections (binary) in plant matrices was conducted using the Jaccard index to assess the similarity of pesticide mixtures in vegetation among sampling sites within the Vinschgau valley. Hierarchical clustering revealed some obvious clusters of Upper and Lower Vinschgau sites whereas mid Vinschgau sites did not form specific clusters. clusters may result from differing application recommendations in the respective valley regions, hence altering the composition of pesticides mixtures along the valley.



Supplementary Figure 3 *Hierarchical clustering using Jaccard Distance. Sites of Upper (UV) are coloured green, Mid (MV) blue and Lower Vinschgau (LV) red.*

Principal Component Analysis (PCA) and regression analysis

Since CUP sum concentration data was found to be not normally distributed (i.e., ranging orders of magnitudes), it was log-transformed prior to PCA [8]. PCA results in a simplification of the underlying, high-dimensional dataset by extracting linear combinations of the original, correlated variables, so-called principal components (PCs). Briefly, PCs provide a reduced number of dominant, uncorrelated components that explain the majority of variability in the underlying data, typically more than 95 %. The first PC hereby explains the largest part of the variance, the second PC most of the remaining variance, until PCs account for the total variance. Since PCs are uncorrelated, issues of correlated predictor variables (i.e., multicollinearity), which can violate regression assumptions, are circumvented. The first two PCs are used as axes of the resulting biplot representing relation of individual sampling sites (points) and original variables (vectors) in the reduced, two-dimensional space. Hereby, (i) the longer a vector is and the more parallel it is orientated towards an axis, the higher is its contribution to this PC, and (ii) the smaller the angle between two vectors, the higher their positive correlation, while negatively correlated variables point in opposite directions. Proximity of points indicates high similarity in their attributes and their ordination along vectors represents the influence of the respective variable on this site [9]. Following PCA, similarities between sampling sites were investigated by cluster analysis (k-means) using Euclidean distance measures. Combining PCA and clustering allows the grouping of sampling sites according to similar land cover and CUP contamination as well as the identification of aforementioned spatial attributes that influence CUP contamination [10].



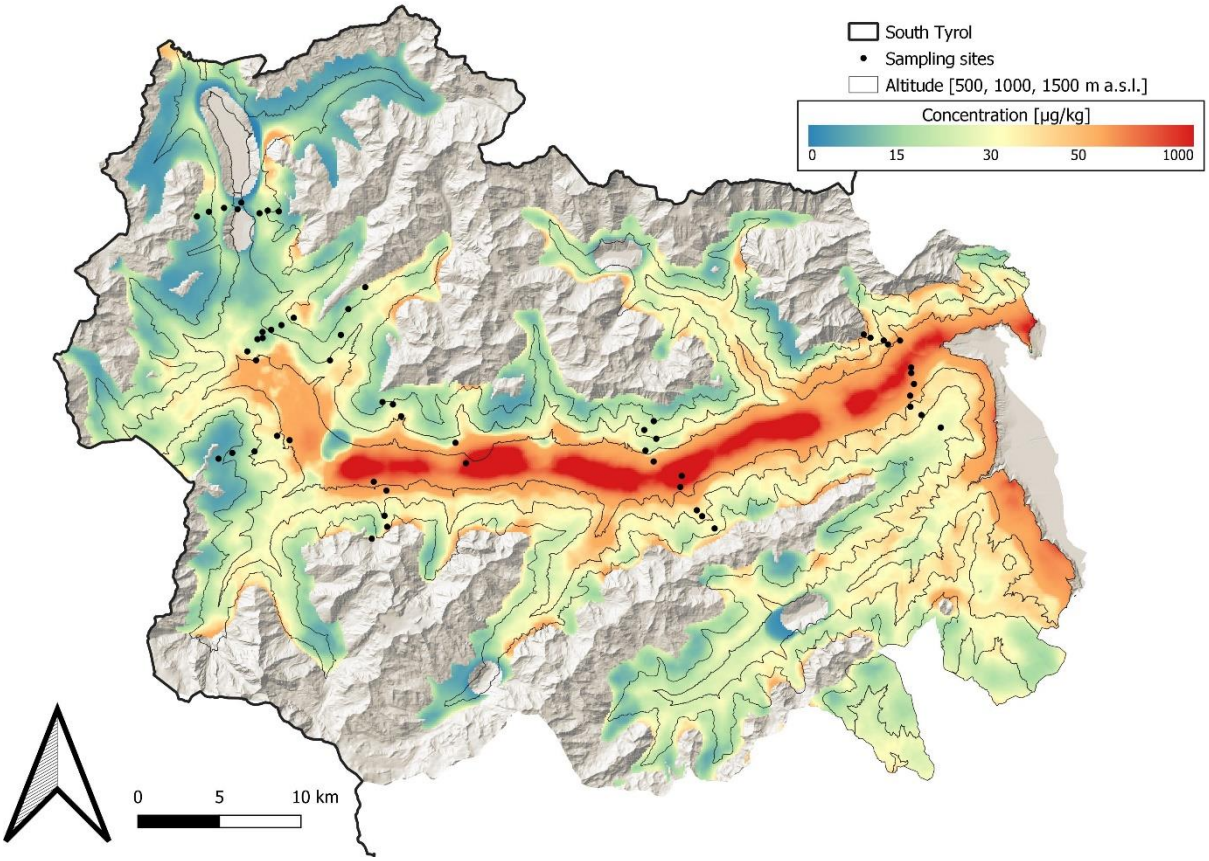
Supplementary Figure 4 *PCA biplot showing the relation between 13 environmental and land cover variables for the 53 sampling sites as represented by the first two principal components (PCs). log.conc = logarithmic pesticide sum concentration of soil and vegetation, h.dif. = height difference to nearest orchard, distance = distance to nearest orchard, alp.grass = alpine grassland and green.urb = green urban areas. Cumulative explained variance: 53.2 % (PC1: 34.9 % and PC2: 18.3 %). Sampling sites were grouped by cluster analysis into four clusters.*

The combination of k-means cluster analysis with PCA showed four significantly distinct clusters among sampling sites, which was primarily defined by the site locations within the landscape (Supplementary Figure 3). The main differences where the immediate vicinity to orchards and resulting high pesticide contamination (cluster 2); sites in vicinity of urban sources with above-average agricultural land-use (excluding orchards, cluster 1); highly remote sites on alpine slopes with substantially lower pesticide contamination and predominantly natural land cover (cluster 3); and, finally, an intermediate group of sites where aforementioned spatial attributes and pesticide contamination were moderately expressed (cluster 4).

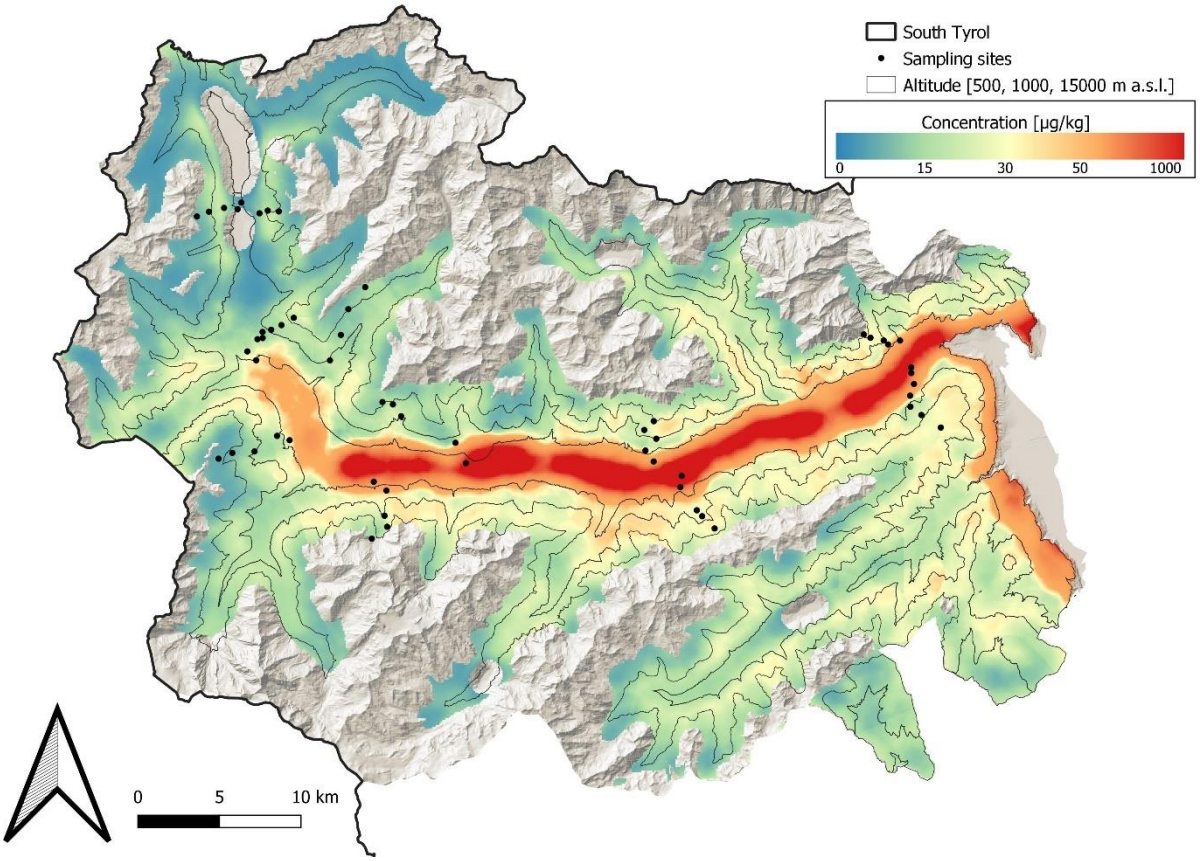
For the principal component regression (PCR), six components were used for the number of CUPs and eight components for concentrations, since they were the minimum number of PCs for which now PCR

accuracy improvements (based on R^2 estimates) could be reached. PCR were validated using 10-fold line segment cross validation with the RMSEP as the validation metric. The final model accuracy was expressed via Pearson's R-squared. For the PCR prediction within the whole Vinschgau, 306,520 landmark points were distributed regularly in a 100 m grid. For all points, environmental variables and land cover attributes were derived in the same way as for the actual sampling points (see 2.4.2). Finally, PCR predicted the estimated CUP numbers and sum concentrations for all aforementioned points [11, 12]. Of the 306,520 points for prediction, only those were used whose spatial attributes were in accordance with the real sampling points in a range of ± 5 % (e.g. < 2,400 m a.s.l.), because model accuracy degraded substantially for points outside the parametrized room. As a result, PCR resulted in the prediction of CUP number and sum concentrations (Supplementary Figure 4) for 119,834 points (1,198 km^2) distributed regularly throughout the Vinschgau.

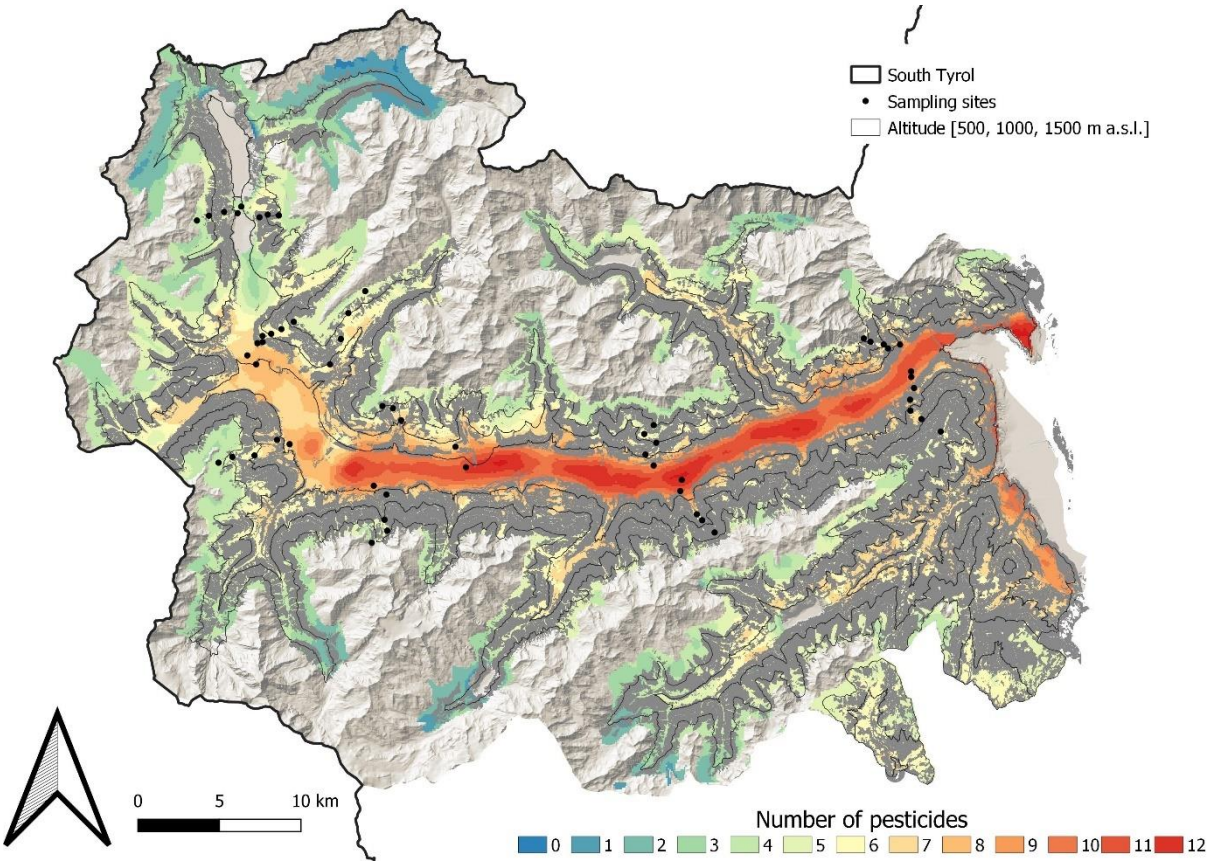
The R packages {corrplot} [13], {NbClust} [14] and {factoextra} [15] were used for PCA and clustering of sampling sites and the package {pls} [16] for PCR. Mapping of PCR results was conducted in QGIS 3.28.1 [17].



Supplementary *Figure 5* *CUP residue sum concentration in soil and vegetation: Predicted CUP sum concentration in the Vinschgau valley based on detected pesticide concentrations along the eleven sampling transects as sum of soil and vegetation samples using principal component regression (PCR). Transect sites shown as black dots.*



Supplementary Figure 6 *CUP residue sum concentration in vegetation: Predicted CUP sum concentration in the Vinschgau valley based on detected pesticide concentrations along the eleven sampling transects in vegetation samples only using principal component regression (PCR). Transect sites shown as black dots.*



Supplementary Figure 7 *Prediction of regression analysis excluding forests (dark grey) as prediction applies primarily to open non-target habitat, e.g., meadows.*

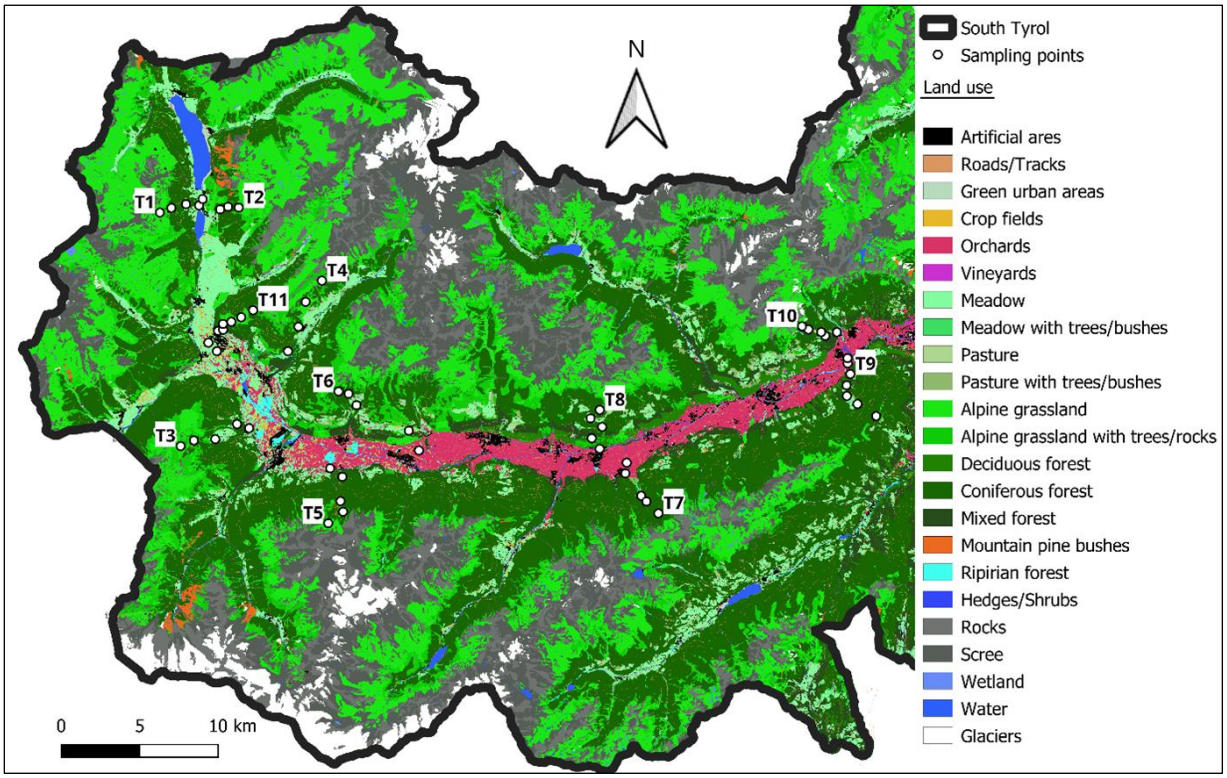
Additional pesticide and environmental parameters

Physico-chemical properties of detected pesticides (vapour pressure (vp [mPa]), Henry's Law Constant (K_H [Pa m^3 mol^{-1}])) as well as ecotoxicological threshold levels were obtained from the Pesticide Properties DataBase [18] of the University Hertfordshire. Pesticides with vp > 5 mPa and K_H > 100 Pa m^3 mol^{-1} were considered volatile compounds, pesticides with vp < 1.3 x 10^{-1} mPa and K_H < 0.1 as non-volatile (Supplementary Table 7). Volatility constants in between these range indicate a weak to moderate volatility [18, 19, 20]. Of the pesticides detected at high altitudes, only pendimethalin (H, T1_4: 2318 m a.s.l.) with a K_H of 1.273 Pa m^3 mol^{-1} and a vp of 3.34 mPa can be attributed a moderate volatility. To all other 27 detected substances in the Vinschgau, no or low volatility would be ascribed (Supplementary Tables 2 & 3). General authorization status of detected pesticides as well as their approval for non-commercial use was determined from the database for plant protection products of the Health Department Italy [21].

Apple cultivation intensity

The data basis for the determination of land use and land cover (LULC) in the Vinschgau were shapefiles of LULC in South Tyrol [22] kindly provided by Erich Tasser (EURAC, Bolzano). Supplementary Figure 5 depicts the relevant regions of the Vinschgau where the sampling transects were located with respective land cover (Supplementary Figure 5). The following LULC types were distinguished: artificial areas, roads, green urban areas, crop fields, orchards, vineyards, meadows, pastures, alpine grasslands, forest, mountain pine bushes, riparian forests, hedges/shrubs, rocks/scree, wetland and inland waters as well as glaciers. To increase explanatory power of variables in subsequent statistical analysis, crop fields and vineyards were combined as agriculture (denoting all agricultural areas despite apple orchards) and forest, mountain pine bushes, riparian forests and hedges/shrubs as vegetation. Mapping and analysis were performed using QGIS 3.28.1 [17].

Buffers of 500, 1000 and 2500 m around each sampling point were tested in principal component analysis (PCA) for relevance of land use data. Since the pesticide monitoring revealed the large distances pesticides may cover by drift, those large buffers were chosen. A buffer of 1,000 m was shown to provide highest explanation of variation in variables in the subsequent principal component analysis (principal component 1 (PC1) = 35 %, principal component 2 (PC2) = 18 %). Hence, a radius of 1,000 m was considered for land use specification around each sampling spot.



Supplementary Figure 8 *Land use and land cover in the Vinschgau with sampling points of the 11 transects (Land use and land cover data modified from [22]).*

Altitude of sampling sites, height differences and distances of sampling sites to the nearest apple orchard were determined with Google Earth [23] as listed in Supplementary Table 1.

The number of CUP detections in soil ($r_s = 0.32$, $p_S = 0.0099$) and in vegetation ($r_s = 0.58$, $p_S < 0.0001$) as well as their sum concentrations (soil: $r_s = 0.29$, $p_S = 0.0182$, vegetation: $r_s = 0.44$, $p_S = 0.0005$) was significantly positively correlated with apple cultivation intensity (percent area used for apple cultivation in 1 km radius around transect site).

Supplementary Tables

Supplementary Table 1 *List of 11 transects with individual sampling sites (n=53), their altitude and distance and height difference (for T1 to valley floor) to nearest apple orchards. PG = playground samples. Apple area = area used for apple cultivation in 1 km radius around transect site [%], Height differences were determined as difference between the altitude of the respective site and the nearest orchard as indicated in the Land Use and Land Cover Data* [22].

Transect	Date of sampling	Sampling Points	Altitude [m a.s.l.]	Distance to orchard [m]	Height difference [m]	Apple area [%]
T1	11.05.2022	T1_1	1461	7108	284	0.00
		T1_2	1725	7318	548	0.00
		T1_3	2040	7465	863	0.00
		T1_4	2318	7467	1141	0.00
T2	11.05.2022	T2_1	1476	6604	299	0.01
		T2_2	1712	6822	535	0.10
		T2_3	1985	7047	808	0.00
		T2_4	2223	7158	1046	0.00
T3	11.05.2022	T3_0_PG	915	50	0	8.87
		T3_1	1198	694	292	3.64
		T3_2	1474	2370	568	0.04
		T3_3	1814	3644	908	0.00
		T3_4	2130	4559	1224	0.00
T4	11.05.2022	T4_1	1470	1543	384	0.17
		T4_2	1746	3153	660	0.14
		T4_3	1997	4723	911	0.00
		T4_4	2300	6445	1214	0.00
T5	10.05.2022	T5_1	939	1	0	33.37
		T5_2	1236	658	256	11.84
		T5_3	1577	1941	597	0.00
		T5_4	1885	2615	905	0.00
		T5_5	2064	3270	1084	0.00
T6	10.05.2022	T6_0	962	20	0	61.41
		T6_1	1225	833	250	6.66
		T6_2	1510	2105	594	0.49
		T6_3	1927	2877	1011	0.00
		T6_4	2065	3192	1149	0.00
T7	10.05.2022	T7_0_PG	629	90	0	28.71
		T7_1	883	0	0	47.49
		T7_2	1195	1257	235	0.00
		T7_3	1310	1729	350	0.00
		T7_4	1840	2760	880	0.00
T8	10.05.2022	T8_1	1056	706	420	7.47
		T8_2	1300	1665	664	0.00
		T8_3	1730	1993	1094	0.00
		T8_4	2000	2554	1364	0.00

T9	09.05.2022	T9_0 _PG	517	20	0	42.88
		T9_1	880	535	343	13.85
		T9_2	1145	1103	608	0.00
		T9_3	1416	1854	879	0.00
		T9_4	1765	3216	1228	0.00
T10	09.05.2022	T10_1	901	280	185	15.86
		T10_2	1239	1008	523	3.68
		T10_3	1529	1255	813	0.12
		T10_4	1883	2085	1167	0.00
		T10_5	2162	2531	1446	0.00
T11	08.05.2022	T11_0	989	20	0	11.72
		T11_1	1156	667	167	3.10
		T11_2	1221	501	232	2.72
		T11_3	1357	859	368	1.74
		T11_4	1550	1029	561	0.71
		T11_5	1820	1616	831	0.00
		T11_6	2175	2058	1186	0.00

Supplementary Table 2 *Overview of number and quantity of detected pesticide residues in the 53 soil samples with respective degradation half-life from soil field studies ($DT_{50,field}$), Kfoc and Kfoc range, Henry's Law Constant (K_H) and vapour pressure (values extracted from respective EFSA regulatory dossiers) as well as additional qualification of bee dangerous substances and those not listed in the guidelines for integrated apple cultivation of AGRIOS [24]. Minimum (min.), maximum (max.) and mean (Ø) concentrations (conc.) are given for samples with concentrations > limit of quantification (LOQ). X samples above LOD but below LOQ (see Supplementary Table 4) CUPS that re recognised as systemic or with systemic properties are marked with an *.*

CUP	Number of samples with detection	Thereof <LOQ	Min. conc. [µg kg^{-1}]	Max. conc. [µg kg^{-1}]	Ø conc. [µg kg^{-1}]	$DT_{50,field}$ range [d]	Kfoc, [ml g^{-1}]	Kfoc range [ml g^{-1}]	K_H [Pa m^3 mol^{-1}]	Vapour pressure [mPa]	Comment
Insecticides											
Acetamiprid *	2	0	0.31	0.44	0.37	0.8 – 5	107	71-138	5.3×10^{-8}	1.7×10^{-4}	
Chlorantraniliprole *	3	0	0.42	2.23	1.13	123 – 561	301,4	180-539	3.2×10^{-9}	6.3×10^{-9}	bee dangerous
Clothianidin *	1	0	1.05	1.05	1.05	13 – 305	160	84-345	2.9×10^{-11}	NA	
Etofenprox	3	0	0.33	1.66	0.95	7 – 25	-	8548-14923†	1.4×10^{-2}	8.1×10^{-4}	bee dangerous
Imidacloprid *	2	2	X	X	X	104 – 228	225	109-411†	1.7×10^{-10}	4.0×10^{-7}	not in AGRIOS
Methoxyfenozide *	21	13	0.39	10.70	2.86	39 – 133	231	132-365	1.6×10^{-4}	1.3×10^{-2}	
Pirimicarb *	1	0	1.05	1.05	1.05	9	290	45-730	3.3×10^{-5}	4.3×10^{-1}	
Thiacloprid *	2	1	1.69	1.69	1.69	6 – 17	615	393-870	4.8×10^{-10}	3.0×10^{-7}	
Fungicides											
Azoxystrobin *	3	3	X	X	X	121 – 262	423	207-594	7.4×10^{-9}	1.1×10^{-7}	not in AGRIOS
Boscalid*	6	4	2.97	23.01	12.99	169 – 312	772	507-1110	5.2×10^{-5}	7.2×10^{-4}	
Cyflufenamid	1	1	X	X	X	10 – 91	1595	1000-2354	2.8×10^{-2}	3.5×10^{-2}	
Difenoconazole *	1	0	1.23	1.23	1.23	20 – 265	3760	400-7730	9.0×10^{-7}	3.3×10^{-5}	
Fluazinam	13	9	0.53	3.69	2.13	21 – 34	1958	1705-2316	5.9×10^{-2}	1.7×10^{-2}	
Fluopyram *	1	1	X	X	X	93 – 145	279	233-400	3.0×10^{-5}	1.2×10^{-3}	
Myclobutanil *	2	2	X	X	X	9 – 58	517	226-920	4.3×10^{-4}	2.0×10^{-1}	
Penconazole *	7	5	1.36	3.32	2.34	67 – 115	2205	786-4120	6.6×10^{-4}	3.7×10^{-1}	
Pyraclostrobin *	4	3	0.22	0.22	0.22	5 – 181	9315	4240-12000	5.3×10^{-6}	5.5×10^{-3}	
Trifloxystrobin	8	1	0.41	3.30	0.88	1 – 3	2287	1642-3745	2.3×10^{-3}	3.4×10^{-3}	
Herbicides											
Flazasulfuron *	1	0	0.83	0.83	0.83	2 – 18	283	59-768	2.6×10^{-6}	1.3×10^{-2}	not in AGRIOS
MCPA *	1	0	5.64	5.64	5.64	25	58	9.4-157	1.5×10^{-5}	4.0×10^{-1}	
Metazachlor	1	0	1.77	1.77	1.77	3 – 21	137	53.8-220	5.9×10^{-5}	9.3×10^{-2}	not in AGRIOS
Metolachlor-S *	1	0	0.28	0.28	0.28	11 – 31	200	112-368	2.4×10^{-3}	1.7×10^{-0}	not in AGRIOS
Napropamide *	1	0	5.18	5.18	5.18	31 – 127	649	208-1593	8.1×10^{-5}	2.2×10^{-2}	not in AGRIOS

Supplementary Table 3 *Overview of number and quantity of detected pesticide residues in the 53 vegetation samples with respective degradation half-life from soil field studies ($DT_{50,field}$), Kfoc and Kfoc range, Henry's Law Constant (K_H) and vapour pressure (values extracted from respective EFSA regulatory dossiers) as well as additional qualification of bee dangerous substances and those not listed in the guidelines for integrated apple cultivation of AGRIOS [24]. Minimum (min.), maximum (max.) and mean (Ø) concentrations (conc.) are given for samples with concentrations > limit of quantification (LOQ). X samples above LOD but below LOQ (see Supplementary Table 4). CUPS that re recognised as systemic or with systemic properties are marked with an *.*

CUP	Number of samples with detection	Thereof <LOQ	Min. conc. [µg kg^{-1}]	Max. conc. [µg kg^{-1}]	Ø conc. [µg kg^{-1}]	$DT_{50,field}$ range [d]	Kfoc, [ml g^{-1}]	Kfoc range [ml g^{-1}]	K_H [Pa m^3 mol^{-1}]	Vapour pressure [mPa]	Comment
Insecticides											
Acetamiprid *	6	0	0.13	36.12	7.37	0.8 – 5	107	71-138	5.3×10^{-8}	1.7×10^{-4}	
Etofenprox	11	3	1.37	228.51	32.34	7 – 25	-	8548-14923†	1.4×10^{-2}	8.1×10^{-4}	bee dangerous
Flonicamid *	1	1	X	X	X	0.7 – 3	5,9	2.5-8.7†	4.2×10^{-8}	9.4×10^{-4}	bee dangerous
Methoxyfenozide *	24	1	0.7	1370.85	65.06	39 – 133	231	132-365	1.6×10^{-4}	1.3×10^{-2}	
Pirimicarb *	4	1	0.44	1.21	0.69	9	290	45-730	3.3×10^{-5}	4.3×10^{-1}	
Spirotetramat *	5	0	1,47	2,72	0,87	0.3 – 1	281	159-435	7.0×10^{-8}	5.6×10^{-6}	bee dangerous
Thiacloprid *	1	0	0.79	0.79	0.79	6 – 17	615	393-870	4.8×10^{-10}	3.0×10^{-7}	
Fungicides											
Azoxystrobin *	1	1	X	X	X	121 – 262	423	207-594	7.4×10^{-9}	1.1×10^{-7}	not in AGRIOS
Cyflufenamid	10	5	2.20	9.38	5.18	10 – 91	1595	1000-2354	2.8×10^{-2}	3.5×10^{-2}	
Difenoconazole *	5	2	4.93	103.91	43.43	20 – 265	3760	400-7730	9.0×10^{-7}	3.3×10^{-5}	
Fluazinam	52	0	3.45	612.21	43.52	21 – 34	1958	1705-2316	5.9×10^{-2}	1.7×10^{-2}	
Fluopyram *	1	1	X	X	X	93 – 145	279	233-400	3.0×10^{-5}	1.2×10^{-3}	
Penconazole *	35	24	2.46	59.55	12.79	67 – 115	2205	786-4120	6.6 x 10-4	3.7 x 10-1	
Pyrimethanil	2	1	14.19	14.19	14.19	5 – 35	419	75-751†	2.2 x 10-3	1.1	
Trifloxystrobin	52	0	0.15	924.50	20.88	1 – 3	2287	1642-3745	2.3 x 10-3	3.4 x 10-3	
Herbicides											
Flazasulfuron *	1	0	7.92	7.92	7.92	2 – 18	2 – 18	283	2.6×10^{-6}	1.3×10^{-2}	not in AGRIOS
MCPA *	1	0	39.21	39.21	39.21	25	25	58	1.5×10^{-5}	4.0×10^{-1}	
Pendimethalin *	5	5	X	X	X	40 – 187	40 – 187	13792	1.27	3.34	

Supplementary Table 4 *Target analytes of the HPLC-MS/MS method with specified chemical group and limits of detection (LOD) and quantification (LOQ) in soil and vegetation for the 97 CUPs. Their recommendation in integrated apple farming in South Tyrol is additionally indicated (according to AGRIOS [24]). Detected pesticides are highlighted in grey.*

Analyte	Chemical group	LOD	LOQ	LOD	LOQ	apple cultivation
		Soil		Vegetation		
Insecticides		[µg kg^{-1}]				
Acetamiprid	Neonicotinoid	0.005	0.014	0.01	0.03	x
Clothianidin	Neonicotinoid	0.16	0.48	0.14	0.44	
Cyantraniliprole *	Anthranillic diamide	0.10	0.30	0.09	0.28	
Dimethoate	Organophosphates	0.02	0.05	0.02	0.06	
Etofenprox	Phenol ether	0.02	0.05	0.01	0.04	x
Fenoxycarb	Carbamates	0.03	0.09	0.04	0.12	
Fenpyroximate *	Pyrazole	0.01	0.02	0.01	0.04	
Flonicamid	Pyridine carboxamides	0.34	1.01	0.41	1.23	x
Flupyradifurone *	Butenolides	0.07	0.20	0.08	0.25	x
Hexythiazox *	Thiazolidinones	0.01	0.02	0.01	0.04	x
Imidacloprid	Neonicotinoid	0.15	0.81	0.27	0.83	
Indoxacarb	Indoxacarb	0.2	0.5	0.14	0.43	x
Methiocarb	Carbamates	0.01	0.03	0.02	0.05	
Methoxyfenozide *	Carbohydrazines		0.03	0.09	0.03	0.09
Pirimicarb	Carbamates	0.003	0.008	0.06	0.17	x x
Pymetrozine	Pyridine azomethines	0.06	0.20	0.02	0.05	
Spinosyn A	Spynosyns	0.03	0.09	0.06	0.17	x
Spinosyn D	Spynosyns	0.11	0.32	0.12	0.35	x
Spirotetramat *	Tetramic acids		0.03	0.09	0.03	0.09
Sulfoxaflor *	Sulfoximine	0.06	0.17	0.04	0.11	x x
Tebufenozide *	Diacylhydrazines	0.02	0.05	0.03	0.09	x
Thiacloprid	Neonicotinoid	0.01	0.03	0.01	0.02	
Thiamethoxam	Neonicotinoid	0.01	0.03	0.02	0.06	
Fungicides						
Azoxystrobin	Strobilurines	0.006	0.018	0.01	0.02	
Benalaxyl	Acetylalanines	0.004	0.013	0.01	0.04	
Bixafen	Pyrazole Carboxamide	0.03	0.10	0.02	0.06	
Boscalid	Pyridine carboxamides	0.1	0.3	0.14	0.43	x
Cyazofamid	Cyano-imidazole	0.05	0.14	0.06	0.19	
Cyflufenamid	Amidoximes	0.04	0.12	0.03	0.09	x
Cymoxanil	Ureas	0.1	0.3	0.17	0.51	
Cyprodinil	Anilo-pyrimidin	0.17	0.51	0.14	0.42	x
Difenoconazole	Triazoles	0.1	0.4	0.14	0.44	x
Dimethomorph	Cinnamic Acid Amides	0.05	0.14	0.04	0.12	
Dimoxystrobin	Strobilurine	0.01	0.03	0.02	0.05	

Fungicides						
Epoxiconazole	Triazoles	0.05	0.15	0.04	0.13	
Fenpropimorph	Morpholine	0.11	0.34	0.16	0.47	
Fluazinam	Phenylpyridilanilines	0.1	0.2	0.1	0.29	x
Fludioxonil	Phenylpyrroles	0.3	0.9	0.21	0.64	x
Fluopicolid	Pyridinylmethyl-benzamides	0.01	0.04	0.02	0.05	
Fluopyram	Pyridine carboxamides	0.01	0.03	0.01	0.04	
Iprovalicarb	Carbamates	0.01	0.03	0.04	0.13	
Kresoxim methyl	Strobilurine	0.1	0.4	0.1	0.32	
Mandipropamid	Mandelic Acid Amides	0.02	0.07	0.02	0.08	
Metalaxyl	Acetylalanines	0.01	0.03	0.01	0.02	
Metconazole	Triazoles	0.06	0.18	0.11	0.33	
Metrafenone	Benzophenone	0.05	0.15	0.05	0.15	
Myclobutanil	Triazoles	0.04	0.11	0.04	0.12	x
Paclobutrazol	Triazoles	0.09	0.28	0.07	0.23	
Penconazole	Triazoles	0.03	0.08	0.04	0.13	x
Pencycuron	Phenylureas	0.05	0.16	0.02	0.05	
Picoxystrobin	Strobilurine	0.02	0.05	0.02	0.06	
Prochloraz	Imidazoles	0.07	0.20	0.1	0.29	
Propamocarb	Carbamates	0.002	0.05	0.003	0.01	
Proquinazid	Quinolines	0.03	0.10	0.02	0.05	
Prothioconazole *	Triazoles	0.03	0.09	0.01	0.04	
Pyraclostrobin	Pyridine carboxamides	0.03	0.09	0.01	0.04	x
Pyrimethanil	Anilinopyrimidines	0.07	0.20	0.08	0.24	x
Spiroxamine	Spiroacetals	0.03	0.08	0.01	0.03	
Tebuconazole	Triazoles	0.14	0.43	0.12	0.37	
Trifloxystrobin	Strobilurines	0.02	0.05	0.01	0.03	x
Herbicides						
2,4-D	Auxins	6.37	19.11	6.8	20.5	
Aminopyralid	Auxins	0.1	0.3	0.14	0.42	
Bentazone	Benzothiadiazinone	0.1	0.3	0.12	0.35	
Bromoxynil	Benzonitriles	0.4	1.2	0.37	1.13	
Carfentrazone-ethyl	Triazolinones	0.2	0.7	0.28	0.86	x
Chloridazon	Pyridazones	0.02	0.06	0.06	0.19	
Chlortoluron	Phenylureas	0.02	0.06	0.02	0.06	
Clomazone	Oxazolidinones	0.03	0.09	0.02	0.06	
Diflufenican	Phenoxynicotinanilides	0.01	0.03	0.02	0.06	
Dimethenamid-P	Chloroacetamide	0.01	0.03	0.02	0.05	
Ethofumesate	Benzofurans	0.1	0.3	0.15	0.44	
Flazasulfuron	Sulfonylureas	0.05	0.14	0.04	1.2	
Florasulam	Triazolpyrimidine sulfonanilides	0.07	0.21	0.12	0.38	
Flufenacet	Oxyacetanilides	0.01	0.05	0.01	0.03	
Fluroxypyr	Auxins	3.8	11.4	3.7	11.1	
Flurtamone	Furanones	0.01	0.03	0.01	0.03	

Herbicides						
Foramsulfuron	Sulfonylureas	0.2	0.6	0.28	0.86	
Isoproturon	Phenylureas	0.02	0.06	0.02	0.07	
Lenacil	Uracil derivates	0.1	0.4	0.19	0.57	
MCPA	Phenoxy compound	1.3	3.8	1.91	5.78	x
Metamitron	Triazinones	0.06	0.20	0.1	0.3	
Metazachlor	Organochlorines	0.01	0.03	0.005	0.01	
Metobromuron	Phenylureas	0.01	0.04	0.04	0.13	
Metolachlor-S	Chloroacetamide	0.02	0.05	0.01	0.04	
Metsulfuron-methyl	Sulfonylureas	0.30	0.80	0.16	0.5	
Napropamide *	Amides	0.01	0.03	0.01	0.04	
Pendimethalin	Dinitro-anilines	0.12	0.36	0.003	0.01	x
Picloram	Pyridines	5.60	17.00	7.1	21.5	
Propaquizafop	Quinoxalines	0.01	0.04	0.01	0.04	x
Propyzamide	Benzamides	0.03	0.06	0.04	0.11	x
Prosulfocarb	Thiocarbamates	0.02	0.05	0.01	0.02	
Quinmerac	Quinolinecarboxylic acids	0.03	0.09	0.03	0.09	
Quizalofop	Aryloxphenoxypropionates	2.91	8.83	2.28	6.92	x
Terbuthylazine	Chloro-s-triazines	0.02	0.06	0.01	0.02	
Tribenuron-methyl	Sulfonylureas	0.02	0.06	0.03	0.08	
Tritosulfuron	Sulfonylureas	0.5	1.6	0.4	1.2	

* Pesticides dissolved separately in MeCN (400 mg L^{-1}) to single substance stock solutions. All other pesticides were purchased as multi-component solutions (100 mg L^{-1}) from Restek (Bellefonte, USA).

Supplementary Table 5 *Descriptive statistics of pesticide numbers and percentages () of total samples in resulting mixtures in soil and vegetation samples (n = 53).*

CUPs	**Soil**	**Vegetation**
Number of contaminated samples	31 (58.5)	52 (98.1)
No CUP residue	22 (41.5)	1 (1.9)
One CUP residue	17 (32.1)	0 (0.0)
Two CUP residues	6 (11.3)	10 (18.9)
Three CUP residues	0 (0.0)	14 (26.4)
Four CUP residues	3 (5.7)	13 (24.5)
Five or more CUP residues	5 (9.4)	15 (28.3)
Ten or more CUP residues	2 (3.8)	2 (3.8)

Supplementary Table 6 *Spearman rank correlation between number of detected CUPs and CUP sum concentration (CUP sum conc.) in soil and vegetation and altitude of sampling sites as well as linear distance to nearest apple orchard (Dist App).*

	soil				**vegetation**			
	CUP Number		CUP sum conc.		CUP Number		CUP sum conc.	
	$\mathbf{r_s}$	p	$\mathbf{r_s}$	p	$\mathbf{r_s}$	p	$\mathbf{r_s}$	p
Altitude	-0.39	<0.018	-0.33	<0.01	-0.57	<0.001	-0.44	<0.001
Dist. App	-0.55	<0.001	-0.49	<0.001	-0.71	<0.001	-0.61	<0.001

Supplementary Table 7 *EFSA peer review conclusions on pesticides' volatility (vapour pressure and Henry's law constant K_H) and aerial transport capability (atmospheric half-life $DT_{50,atm}$) of substances detected at highest sampling sites.*

Pesticide	**Max. detection altitude [m a.s.l.] (site)**	**Height above valley floor [m]**	**Vapour pressure at 20°C [mPa]**	**K_H at 25 °C [Pa m^3 mol^{-1}]**	**$DT_{50,atm}$ [d]**	**EFSA estimate on volatility and transport (peer review)***
Etofenprox (I)	2175 (T11_6)	1145	8.13×10^{-4}	1.36×10^{-2}	0.2	*"low potential for volatilization, due to rapid photochemical transformation environmental concentrations in air and transport through air are considered negligible"*
Methoxyfenozide (I)	2162 (T10_5)	1205	1.33×10^{-2}	1.64×10^{-4}	NA	not considered
Azoxystrobin (F)	2064 (T5_5)	1188	1.10×10^{-7}	7.40×10^{-9}	8.7 - 13.9	*"low potential for volatilization, long-range transport through the atmosphere is not expected"*
Fluazinam (F)	2318 (T1_4)	853	1.72×10^{-2}	5.93×10^{-2}	2 - 163	*"may be relatively persistent in air, expected to have a medium to high potential for volatilization, long-range transport cannot be completely excluded"*
Penconazole (F)	2223 (T2_4)	771	3.66×10^{-1}	6.6×10^{-4}	1.3 - 2	*"very slightly volatile, only limited losses due to volatilisation expected, unlikely to be subject to long-range atmospheric transport, not expected to be present in air for extended time periods or be transported over significant distances"*
Trifloxystrobin (F)	2318 (T1_4)	853	3.40×10^{-3}	2.3×10^{-3}	NA	*"not subject to long range atmospheric transport"*
Pendimethalin (H)	2318 (T1_4)	853	3.34	1.27	0.3	*"rapidly degraded by photochemical processes in the atmosphere, no risk for long-range transport via air"*

* Sources: [18, 25-29]

Geographic conditions and spatial prediction

Supplementary Table 8 *PCA results for the 13 principal components (PC).*

Components	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**	**PC7**
Standard deviation	2.1312	1.5405	1.3591	1.1331	1.0169	0.7995	0.6428
Proportion of variance	0.3494	0.1826	0.1421	0.0988	0.0795	0.0492	0.0318
Cumulative Proportion	0.3494	0.5320	0.6740	0.7728	0.8523	0.9015	0.9333
Components	**PC8**	**PC9**	**PC10**	**PC11**	**PC12**	**PC13**	
Standard deviation	0.5461	0.4785	0.3839	0.3464	0.2648	0.0526	
Proportion of variance	0.0229	0.0176	0.0113	0.0092	0.0054	0.0002	
Cumulative Proportion	0.9562	0.9738	0.9852	0.9944	0.9998	1.0000	

Supplementary Table 9 *Contribution of variables [%] to the first two principal components (PC) of PCA.*

Variable	**PC1**	**PC2**
Altitude	19.03	1.38
Height difference to orchard	16.34	0.06
Distance to orchard	10.55	6.51
Alpine grassland	9.62	7.77
Orchards	9.32	3.30
Agriculture	7.40	12.29
Rock	7.36	0.58
Green urban areas	6.37	16.43
Log. pesticide concentration	5.98	8.32
Meadows	5.81	15.03
Water	1.50	6.46
Vegetation	0.69	20.25
Pastures	0.03	1.62

Effect of wind on pesticide distribution

For comparison of pesticide distribution by wind onto north- and south-facing slopes, T3 – T10 were considered as transects directly opposing each other on valley slopes. T1 and T2 were excluded since they are east-, respectively, west-facing and not directly affected by northern Föhn wind. T11 as additional transect without counterpart was excluded as well. Considering all sampling sites on the included transects, higher pesticide numbers and concentrations were observed on north-facing transects but differences were not statistically significant. This finding can be explained by most of the highly contaminated valley floor samples affected by primary drift from nearby orchards being attributed to north-facing transects (i.e. T3_0, T7_0 & T7_1, T9_0, compare 3.2). Hence, sampling sites on the valley ground (T3_0, T6_0, T7_0, T7_1, T9_0) were excluded for the final north-south comparison because they may bias the evaluation of the impact of the wind direction on the landscape scale pesticide distribution in the Vinschgau. Only transect points on hillsides (n = 33) were considered because they are more exposed to and impacted from wind and its direction. In soil hillside samples, 24 pesticide detections were made on north-facing transects, while a total of 20 detections was made on south-facing transects. In vegetation, 60 detections were made on northern and 64 detections on southern hillsides. Contrary to number of detected pesticides, the total pesticide concentration was higher on south-facing hillsides in soil (137.61 µg kg^{-1}) compared to north-facing sides (soil: 10.23 µg kg^{-1}), while the total concentration in vegetation was higher on north-facing sides (946.67 µg kg^{-1}) compared to south-facing sides (385.31 µg kg^{-1}). These differences between north- and south-facing transects were not statistically significant, neither for number of pesticides (N_{north} = 16, N_{south} = 17, soil: Mann-Whitney U = 146, p = 0.7025, z = -0.38, |r| = 0.07, vegetation: Mann-Whitney U = 131, p = 0.8532, z = -0.19, |r| = 0.03) nor for pesticide sum concentrations (soil: Mann-Whitney U = 123, p = 0.6182, z = -0.50, |r| = 0.09, vegetation: Mann-Whitney U = 146, p = 0.7187, z = -0.36, |r| = 0.06).

Extended butterfly and CUP exposure discussion

Recently, entomologists conducted butterfly studies at 93 sites in South Tyrol and in the Vinschgau valley [1, 30, 31, 32]. They concluded from comparisons of recent butterfly abundance with historical observations that populations were not only impacted in habitats neighbouring apple orchards but also up to 300 m above the valley floor. They found individual abundance, species richness and species diversity to be highest in extensively managed meadows and pastures up to 1,600 m a.s.l., followed by semi-intensively managed meadows and lowest values in apple orchards. Their findings can also be applied to the trends disclosed in the measurement of pesticide residues: Lower complexity of CUP mixtures and lower concentrations in higher altitudes translate into well-established butterfly communities, while significantly higher number and concentrations of CUPs, and especially insecticides, in valley locations may cause the disappearance of butterflies [30]. In more detail, pesticide contamination as assessed in the present pesticide monitoring in the Vinschgau could directly be linked to observed butterfly abundance at several sampling sites [1, 33]. In his 2019 study, Tarmann[1] describes the sampling point T11_4 (*Malettes*: 1550 m a.s.l.), a large, remote meadow, as a *"valuable butterfly meadow with more than 1,000 specimens, 23 different species among which 6 are on the Red List of endangered species"* with *"a mass occurrence never observed before "*[1]. This richness coincides with the nearly CUP-free soil (n = 2) and the only vegetation samples in the entire study without any CUP recorded (n = 0). Similar observations were made in the side valley Matschertal into which transect T4 extends. On the dry meadows and steppe slopes characteristic of this side valley, diverse, intact butterfly communities were observed [1, 33]. The researchers hypothesized that the remote location of this valley, away from apple cultivation, is the reason for being spared from chemical input. The present study's supports this hypothesis by finding the range of contamination in the Matschertal (i.e. sum concentration of sampling points on T4: 5.35 – 6.84 µg kg-1) to be lower than that recorded along all other transects. In contrast, meadows near the municipalities of Eyrs, Laas and Latsch were described as low-quality, degraded habitats with less butterfly abundance and diversity. Additionally, meadows near Latsch had impaired butterfly communities up to altitudes of 300 m above the valley floor; only meadows higher than that hosted butterflies not or just slightly impacted [33]. Pesticide sampling sites could be linked to these meadows. At T5_1 (near Eyrs), T6_0 (near Laas) and T7_0 (playground in Latsch) several pesticides were detected in their highest concentrations. The presented CUP detection data support Huemer's explanation for the drastic decline of butterflies in these valley regions [33]. Tarmann speculated that the Tartscher Bichl (46°40'43" N, 10°33'34" E, 1077 m a.s.l.), a bald, rocky outcrop south-east of Mals, represents the limit up to which pesticide drift from lower valley levels clearly influences butterfly habitats, although no measurements were available [1]. On all other sites north of the Tartscher Bichl, well-established populations were observed [1]. This locational trend of butterfly observations again could be transferred to the sampled transects. Transects T1, T2, T4 and T11 with a low degree of pesticide contamination, and

well-established butterfly populations, lie “behind” (north of) the Tartscher Bichel in the Upper Vinschgau. All other transects with higher CUP detections were located in areas with butterfly populations described as seriously compromised.

Caterpillars are not only directly affected by insecticide residues on plant material that they ingest, but can also reveal reduced development after exposure of plants to low concentrations of herbicides [34]. The herbicides potentially trigger plant defence mechanisms that reduce plant food quality resulting in reduced growth and lower pupation success. Due to the complexity of mixtures and potential indirect and direct effects it is currently not possible to predict the effect of chronic food exposure with CUP mixtures at low, sublethal concentrations on butterfly and moth populations.

Supplementary References

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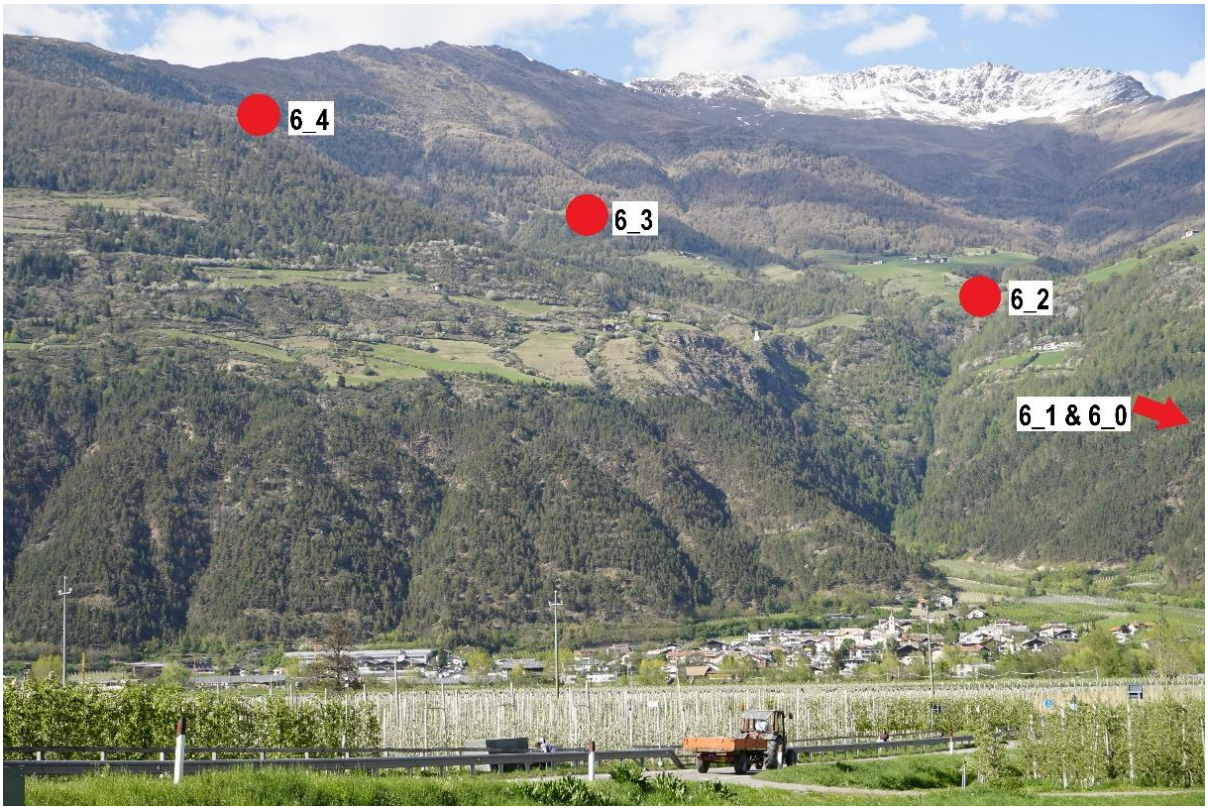
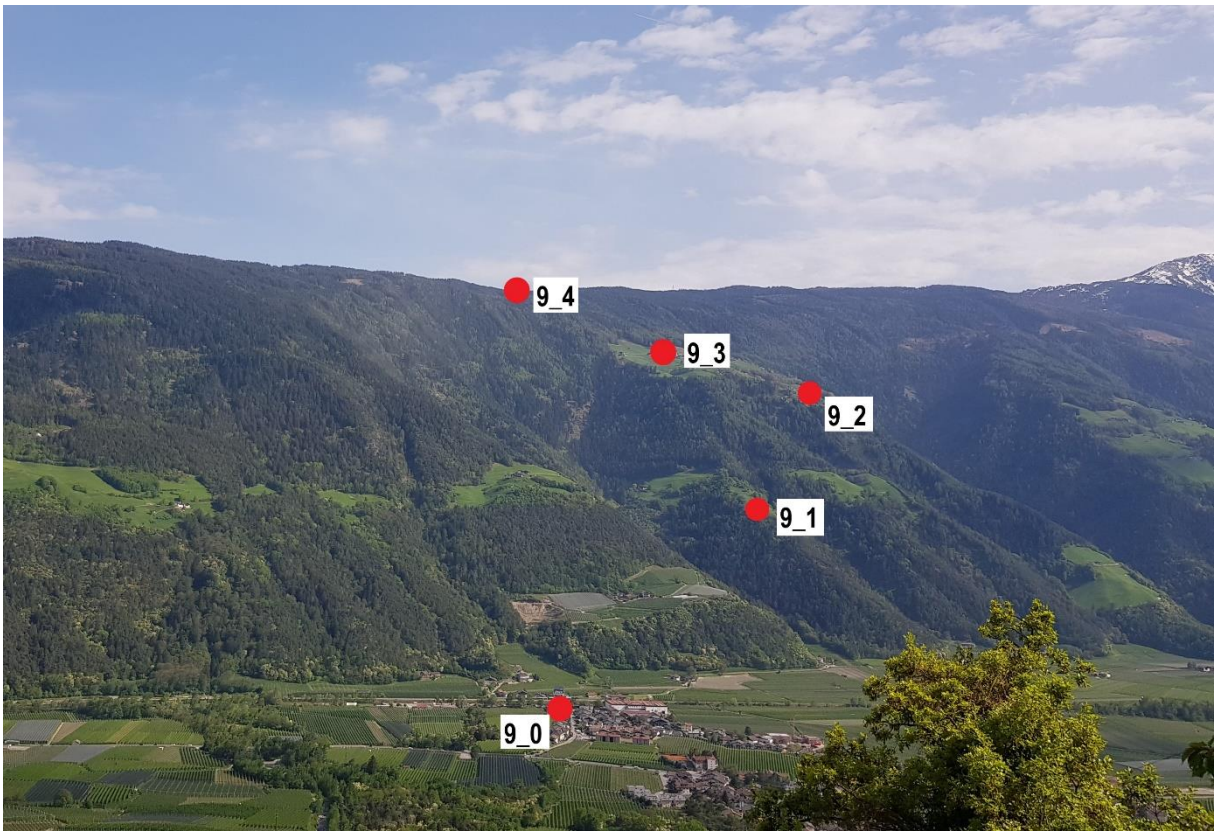
Pictures of selected transects

Supplementary Figure 9 *Transect T1 located at the Reschensee in St. Valtentin a. d. Haide in the Upper Vinschgau (UV) extending from 1,461 to 2,318 m a.s.l. There is no apple cultivation in the valley (picture by Nina Engelhard).*



Supplementary Figure 10 *Transect T6 located near Tanas in the Mid Vinschgau (MV) extending from 962 to 2,065 m a.s.l. with apple cultivation in the foreground (picture by Carsten Brühl).*



Supplementary Figure 11 *Transect T9 located near Rabland in the Lower Vinschgau (LV) extending from 517 to 1,765 m a.s.l. The valley is dominated by apple cultivation (picture by Nina Engelhard).*

Supplementary Figure 12 *View from transect T10 from Lower to Mid Vinschgau along the valley main axis. The river Etsch is visible in the centre, the entire valley bottom is covered by apple plantations. South-facing slopes (right) with steppe and open brush vegetation, North-facing slopes (left) with dense spruce forest (picture by Carsten Brühl).*