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Materia della tesi VITICOLTURA

Effects of biodynamic treatment 500 and 501 in managed vineyards in Switzerland: influences on soil, plants and grape quality

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Abstract

In the past few years, wines produced applying the biodynamic system have received increasing attention. This system, like organic farming, eliminates the use of synthetic chemical fertilizers and pesticides, and replaces these traditional treatments with soil and plant amendments called preparations (500 applied on soil and 501 atomized on plants).

In this thesis we shall analyze whether biodynamic preparations 500 and 501 actually augment the health of soil and plants and the quanta-quality of grapes and wine. Presently, the effects of biodynamic treatments in general and on wine making are still unclear. In 2006, FIBL (Forschungsinstitut für biologischen Landbau) with other partners carried out a 6-year study in four biodynamic farms located in two different regions of Switzerland (three grown with cv. Chasselas and one grown with cv. Riesling & Silvaner).

The experimental design provided for: 1 plot treated with a 500 preparation, 1 plot treated with a 501 preparation, 1 plot treated with a combination of 500 and 501 (Combi), 1 plot, the control plot, no treatment and exclusively organically farmed.

The object of monitoring was the chemical analysis of the soil, the analysis of the structure of aggregates in the soil, and the microbial activity. For the plant part the nutrients in leaf tissue, chlorophyll-index, phyto-alexine, disease pressure, and the weight and length of the shoots.

No differences were noticed in the chemical composition, but over the six-year period the humus percentage was reduced in the biodynamic plots (contrarily to literature), however to this respect there is thesis arguing that this fact may be due to an insufficient supply of manure. In fact in Elfingen the elements K, P increased and presented a higher increase in humus compared to the other sites. Statistical differences were noticed in higher structure of the aggregates in the Combi soil than Control, Elfingen evidenced the highest improvement. This date is relative to the measurements carried out in 2009, but was not confirmed in

2011.

As for the microbial activity, the microbial analysis show differences only in depth and position in the row, only in Elfingen there was an improvement in microbial activity. In order to gather a better view and avoid environmental interferences, each site was individually studied.

Significant results were obtained in the SPAD values and wood weight for the plants treated with 501, in order to gather a better view each site was individually studied and the univariate ANOVA test evidenced an interaction 500x501.

No differences were observed in the nutrient analyses carried out on the leaves, except for Mn, Mg and K.

Disease incidents and severity show no statistical differences except for an statistically interesting high presence of phyto-alexine in the leaves of plants treated with 501. Remarkable but not statistically significant the treatment with 500; the combination of the two treatments yields a higher effect in the plant's immune system.

No statistical differences were observed in the grape harvest yield, in the quality of the must.

Wines for the period 2008-2010 were subjected to standard analysis evidenced no differences except for nearly significant lactic acid, which was higher in Combi plot wines, while malic acid was lower in Combi wines.

Wine sensorial analysis were done with a triangle test and show no differences except for Chasselas 2009.

The organoleptic sensorial blind tests evidence results only in the criteria minerality nose.

Abstract

Negli ultimi anni, i vini prodotti applicando il sistema biodinamico hanno ricevuto crescente attenzione. Questo sistema, come del resto l'agricoltura biologica, elimina l'uso di fertilizzanti chimici di sintesi e pesticidi, e sostituisce i trattamenti tradizionali con ammendanti per terra e piante chiamati preparazioni (500 applicato sul suolo e 501 nebulizzato sulle piante).

In questa tesi analizzeremo se i preparati biodinamici 500 e 501 effettivamente aumentano la salute del suolo e delle piante e la quanti-qualità dell'uva e del vino. Attualmente, gli effetti dei trattamenti biodinamici in generale e sulla produzione del vino sono ancora poco chiari. Nel 2006, FIBL (Forschungsinstitut für biologischen Landbau) con altri partner hanno condotto uno studio della durata di 6 anni in quattro aziende biodinamici situate in due diverse regioni della Svizzera (tre in cui è coltivato il cultivar Chasselas e uno coltivato con il cultivar Riesling e Silvaner).

Il disegno sperimentale prevedeva: 1 terreno trattato con un preparato 500, 1 terreno trattato con un preparato 501, 1 terreno trattato con una combinazione di 500 e 501 (Combi) , 1 terreno di controllo con nessun trattamento ed esclusivamente lavorato con agricoltura biologica.

L'oggetto di monitoraggio è stata l'analisi chimica del suolo, l'analisi della struttura degli aggregati nel suolo, e l'attività microbica. Per la componente pianta i nutrienti nel tessuto della foglia, l'indice di clorofilla, fitoalessine, la pressione provocata dalle malattie, e il peso e la lunghezza dei germogli.

Nessuna differenza è stata notata nella composizione chimica, ma nel corso del periodo di sei anni la percentuale di humus è stata ridotta nei terreni biodinamici (contrariamente alla letteratura), ma a questo proposito esiste una tesi che sostiene che questo fatto può essere dovuto ad un insufficiente apporto di letame. Infatti a Elfingen gli elementi K, P sono aumentati ed hanno presentando un incremento maggiore di humus rispetto agli altri siti. Differenze statistiche sono

state rilevate nella maggiore struttura degli aggregati nel terreno Combi rispetto a quello di Controllo, Elfingen ha evidenziato il maggiore miglioramento. Questi dati sono relativi alle rilevazioni effettuate nel 2009, ma non sono state confermate nel 2011.

Per quanto riguarda l'attività microbica, le analisi microbiche mostrano differenze solo in profondità e posizione nella fila, solo in Elfingen c'è stato un miglioramento dell'attività microbica. Al fine di avere una visione migliore dei dati ed evitare interferenze ambientali, ogni sito è stata studiato singolarmente.

Risultati significativi sono stati ottenuti nei valori del peso del legno e SPAD per le piante trattate con 501, al fine di avere una visione migliore ogni sito è stato studiato individualmente e il test ANOVA ha evidenziato una interazione 500x501.

Non sono state osservate differenze nelle analisi delle sostanze nutritive effettuate sulle foglie, ad eccezione di Mn, Mg e K.

Eventi di malattia e la gravità degli stessi non mostrano differenze statisticamente significative ad eccezione di una elevata presenza statisticamente interessante di fitoalessine nelle foglie delle piante trattate con 501. Notevole ma non statisticamente significativo il trattamento con 500; la combinazione dei due trattamenti produce un effetto superiore nel sistema immunitario della pianta.

Nessuna differenza statistica è stata osservata nel rendimento della vendemmia, nella qualità del mosto .

I vini per il periodo 2008-2010 sono stati sottoposti ad analisi standard non hanno evidenziato nessuna differenza tranne che per un quasi significativo acido lattico, che era maggiore nei vini dei terreni Combi, mentre l'acido malico è stato inferiore nei vini dei terreni Combi.

Le analisi sensoriali dei vini sono state fatte con un test triangolare e non mostrano differenze fatta eccezione del Chasselas 2009. Il test sensoriale organolettico a cieco mostrano evidenza solo nel criterio della mineralità nel naso.

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General Introduction

We need a different approach and method in agriculture; otherwise, our soils will slowly deteriorate. In the alternative agriculture movements, biodynamic methods respond to concerns from farmers about the health of their farms (Steiner 1923), deterring the use of synthetic chemical fertilizers and pesticides. biodynamic is an agronomic approach that emphasizes soil building and high diversity of crops, animals, and wildlife habitat (Koepf et al. 1990), and attempts to create a closed cycle farm where the outputs are minimized. In addition to the soil, biodynamic practices use fermented manure, specific compost, crops, plant matter, and natural pest control. The character of the eight preparations is designated by their ingredients and soil nutrient cycling are supposed to promote photosynthesis and a sustainable complex soil-humus (Koepf et al. 1990).

Scientific evidence for environmental benefits of organic and biodynamic farming has been given in many studies, but the nutritional and sensory qualities have not often been proven in contrast to conventional produce (Christine M Arncken et al. 2012). Organic and biodynamic farming practices are increasingly used worldwide. Further research could support this trend.

In the European wine sector, more than 231.412 hectares of vines are grown organically as of 2010. This represents 5.6 % of total vineyard area (Schweizerische Weinzeitung sept.2013). In Switzerland, the Demeter-certified agricultural area represented 0.39% of the utilized agricultural area (UAA). At the beginning of 2013, there were 30 winemakers, 125ha in total, which represents about 0.56% of all farms with vineyards; but, the number of organic and biodynamic vineyards increases every year.

Growth in biodynamic viticulture has been particularly rapid in France, with 1000ha of wine grapes cultivated biodynamically in 1993, and 15,000ha in 1998 (Meunier 2001). Research on the preparations suggests that they may benefit soil quality and crop quality (Koepf 1993, Reganold 1995); however, the results are mixed.

The study “DOK 21 years comparing system” (Mader et al, 2002) shows a change in the structure of the soil, and higher microbial activity and numbers of earth-worms. The total mass of microorganisms in the organic system of the DOK-trial was 20%-40% higher than in the conventional system with manure, and 60%-85% higher than in the conventional system without manure. The study by Reganold et al. in 1990 confirms this hypothesis; however, the soil nutrient analyses were varied.

In New Zealand, the biodynamically-farmed soils had better structure and tilth, which allowed the development of a seedbed more so than conventionally-farmed soils (Reganold et al., 1993). In general, biodynamically-farmed soils show a higher biological and physical quality as well as significantly greater organic matter content and microbial activity (Reganold et al., 1993). On the same note, studies by Petterson in Sweden and Penfold in Australia compared plots receiving biodynamic sprays with control plots with inconclusive results. Whether these preparations actually augment soil or crop quality is unclear and controversial (Koepf, 1993; Reganold, 1995; Bourgignon & Gabucci, 2000; Carpenter-Boggs et al., 2000; Mäder et al., 2002; Reeve et al., 2005), but it’s proven that organically treated soils supply a privileged habitat for microorganisms and paedo fauna. As the soil’s microecosystem is a key criterion for the fertility of the soil functions and providing for a better plant growth.

Organic agriculture involves targeted humus management. The addition and natural accumulation of organic material should at least replace the humus lost through decomposition. This objective is achieved through the cultivation of grass leys and suitable green manure crops, by limiting the proportion of root crops in the rotation and by incorporating organic matter. (Std BIOSUISSE 2013)

The evidence indicates that an alliance of these systems is crucial for achieving a sustainable agriculture.

Are grapes the ideal crop for studying the differences in soil and fruit quality arising from different types of agricultural management?
The abuse of chemicals in modern viticulture and the need expressed by many operators worried by the deterioration of soils, and the ensuing search of alternative routes, has increased the corpus of data available, offering an important opportunity to effectively study on the field the application of different agricultural management systems.
Active cooperation with the viticulture and oenology markets is a further opportunity to carry out more in depth studies of Biodynamics, a better knowledge of which can play a significant role in the development of a different approach providing the necessary answers and solutions to soil deterioration on one hand, while on the other improving the quality/cost ratio.

Goal of the 6-year study:

The goal of the 6-year study which was requested by farmers, and consequently of this thesis was to analyse the effect of the most important biodynamic preparations 500, 501, and Combi (500+501) on: quality of soil, plant physiology, grapes, and wine quality.
However, it is important to keep in mind that these are only 2 of 8 biodynamic preparations and techniques commonly adopted in biodynamic farming.
The study is the fruit of the cooperation between biodynamic farmers, the Research Institute for organic Agriculture (FIBL, Frick CH), Demeter (BD Label), and several private foundations.

2 Material and Methods

2.1 Research site

Between 2006 and 2011, a field trial on the use of biodynamic preparation was carried out in three different areas of the Swiss-French region (Hauterive, Auvernier, Echandens) and one in the German region (Elfingen) (Fig. 1). During the period spanning between 2006 and 2008, the biodynamic preparations were applied in these areas, however data collection did not begin until 2008.

The different sites for the trials were chosen in order to achieve a greater strength

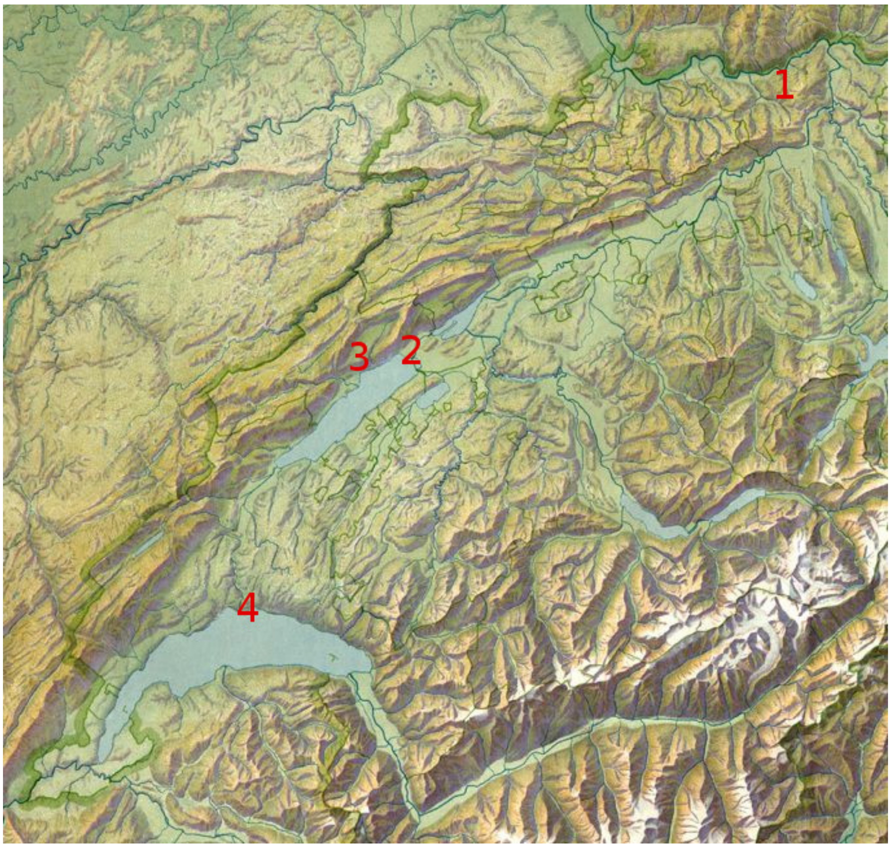

Figure 1: Location of tests areas and arrangement of the experimental plots. 1: Elfingen, Häfliger, 2: Hauterive, Rossel, 3: Auvernier, Henrioud, 4: Echandens, Barilier.

in the research conclusions, as these vineyards differed in many aspects: climatic conditions, soil properties, grapevine cultivars, etc., as well as in the viticulture practices used by each estate, however this choice caused a higher degree of difficulties in the processing of the data.

The cultivar were arranged as follows: Auvernier (Neuchatel) alt 455m, 3.0 ha yard (Vitis vinifera L. cv Chasselas, grafted onto 5BB rootstock). Hauterive (Neuchatel) parcel "Wolf haut" alt 480m, (Vitis vinifera L. cv Chasselas, grafted onto 3309C rootstock). Echandens (Waadt) parcel "le Chapitre" alt 432m (Vitis vinifera L. cv Chasselas, grafted onto 5BB rootstock). Elfingen (Aargau) parcel "Ruegget" alt 513m (Vitis vinifera L. cv Riesling x Silvaner, grafted onto 5BB rootstock).

2.2 Meteorological Parameters

Precipitation (mm) and temperature (°C) were measured by a weather station (Lufft) located near the experimental vineyards. The transmission of values through GSM was performed twice a day, and could be accessed by the Agrometeo webpage (http://www.agrometeo.ch/it/meteorology/datas). The weather conditions are reported in table 1 and displayed on graphs 1 and 2.

Water stress at the beginning of the year and early flowering. Cold July but hot period in August-September.

Year	Elf		Auv-Haut		Ech		Notes
	Temp. (°C)	Precip. (mm)	Temp (°C)	Precip (mm)	Temp. (°C)	Precip. (mm)	
2008	10.1	1358.8	10.4	1033.4	-	-	Alternance wet and very hot with storms, dry at harvest.
2009	10.3	1357.9	10.9	1014.2	12.7	874	Hot and early year, (beginning of buds) precocity harvest
2010	9.3	1247.2	9.8	884.8	10.4	918	Frost in the winter and dry spring followed by a hot summer, season on the average.
2011	10.9	809.4	11.4	880.8	11.9	870.2	Water stress at the beginning of the year and early flowering. Cold July but hot period in August-September.

Table 1: Average temperature 2009-2013 in ºC and precipitation 2009-2013 in mm for the four sites (No data is available for site Ech in 2008).

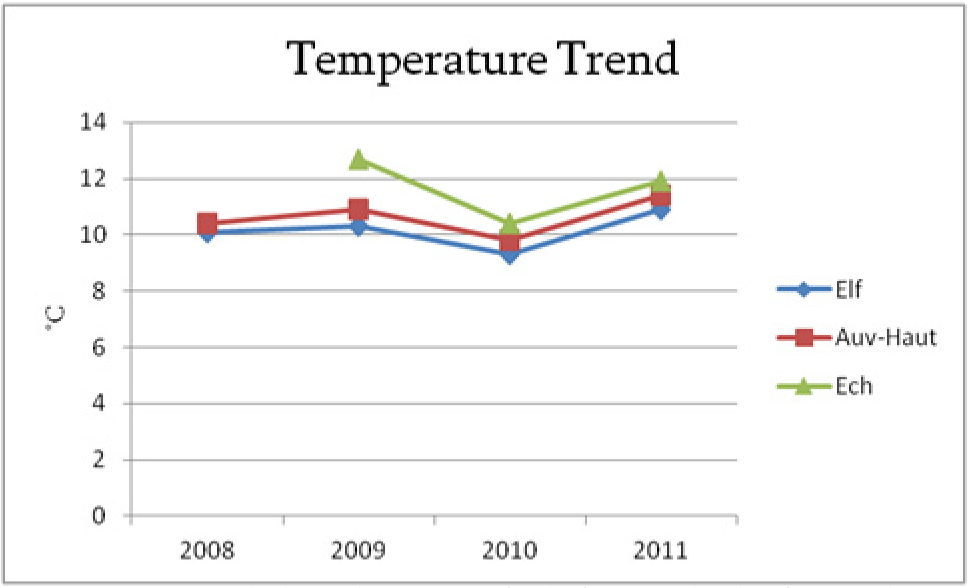

Figure 2: Temperature (°C) trend 2008 - 2011 for the four sites (No data for site Ech in 2008)

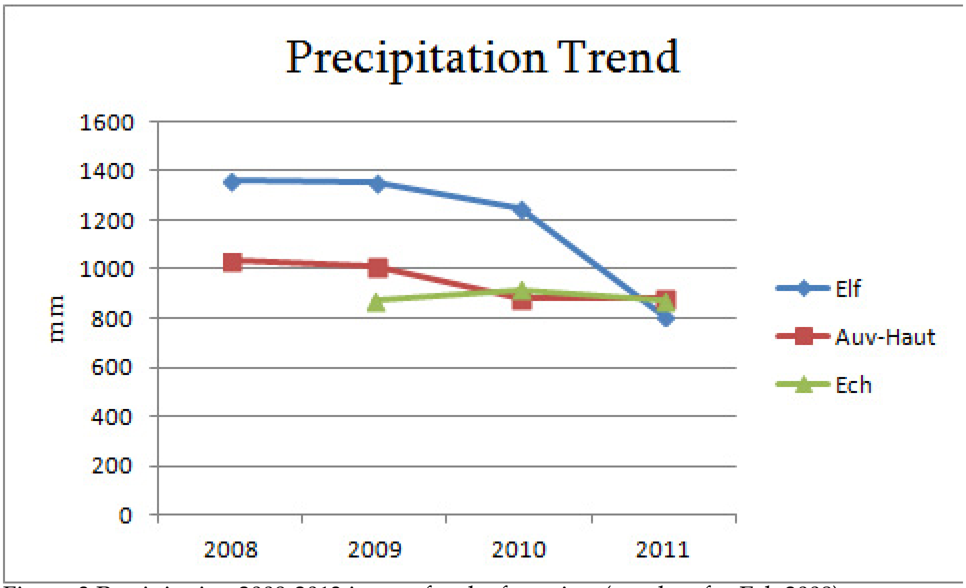

Figure 3:Precipitation 2009-2013 in mm for the four sites (no data for Ech 2008)

2.3 Experimental Design

The experimental treatments consisted of 500 alone, 501 alone, the combination of both, and a control plot where no biodynamic preparations were applied. All management practices were the same in all sub-plots of each site, except for the addition of the preparations to the organic treatments. The exact same 500 and 501 preparations were used in all experimental sites, but their distribution was carried out by each estate.

The application calendar for the biodynamic preparations can be found in *Table 3*. Each treatment was replicated three times in a randomized block design.

All the plot areas were certified biodynamic before the study was initiated:

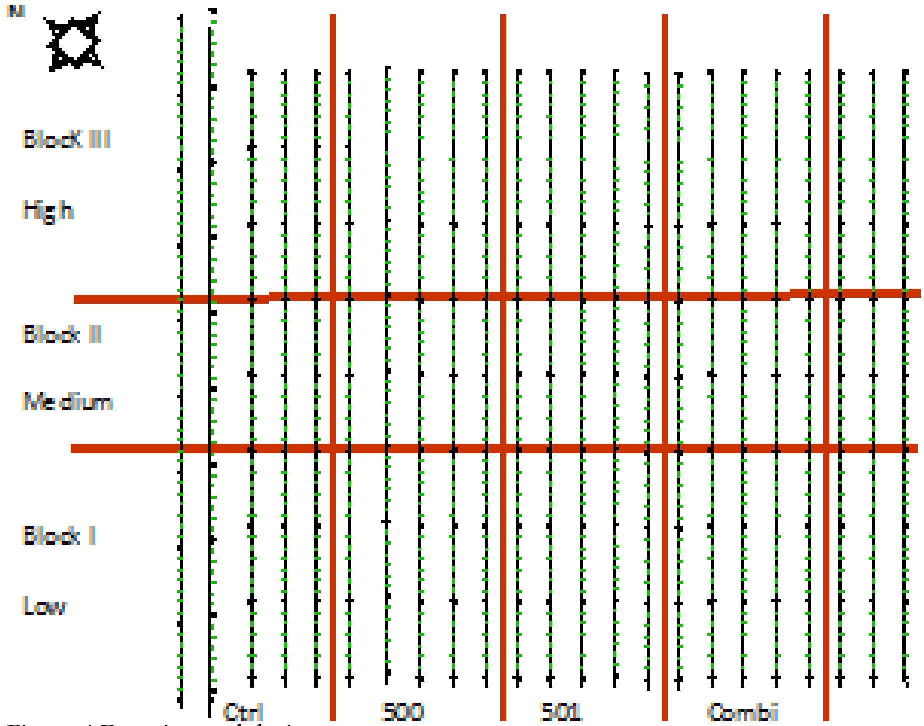

Figure 4:Experimental design.

Elf was certified biodynamic in 2003, Ech was certified in 2000, Haut in 2002, and Auv in 2002. Each plot consisted of 5 rows treated with 500, 5 rows treated with 501(only Auv missing), 5 rows treated Combi, 5 rows treated Control (Figure 2). All vineyards in the examined sites are from 30 to 50 years old. Ech, Auv, and Elf used the Guyot training system, and Haut adopted the Cordon system. The vineyards were planted in a low density (6000 p/He) at Hau, Auv and Elf, and Ech planted with a higher density of 7000 p/he.

None of the four sites used irrigation and no manure was added for the whole trial period in Ech and Auv. In Haut manure was added during 2011, instead 3 t/He biodynamic compost was added. In 2012 in Elf each vine received an organic fertilizer, Biosol (N), in the amount of 50 Kg N total hectare and 7 Kg P hectare. Still in Elf, in the spring of 2013, a mushroom compost was added. The Control plot was designed for biological farming, but it did not provide for organic manure additional treatments. The biodynamic preparations were prepared and delivered by Pierre Masson (www.biodynamie-services.fr) and applied to specific rows. The standard treatments for the four locations were the distribution of 120 gr/ha in April and June, and 4 gr/ha of horn silica in June. Details are shown in *Table 2*.

		April	May	June	July	Aug.	Sept.	Oct.	Nov.
	2008	500P(30th)			501(10th)		501(21st)	501(7th)	
Elf	2009	500P (16th&25th)		501(10th)	501(26th)		501(17th)	500P(29th)	
	2010	500(14th)			501(15th)	501(30th)			
	2008	500(22th)		501(20th)					500(12th)
Auv	2009	500(20th)	501(22nd)					500(14th)	
	2010	500(20th)		501(22nd)				500(14th)	
	2008		500(13th)		501(10th)		501(11th)		501(31st)
Haut	2009		500(4th)	501(4th)		501(14th)			
	2010		500(4th)	501(18th)	500(6th)		501(28th)		
	2008		500(13th)		501(10th)				500 (3rd)
Ech	2009		500(4th)- 501(13th)			501(22nd)			
	2010		500(4th) 500(19th)	501(9th)					

Table 2: Day and month of treatment with preparation 500 and 501 in 2008-2009-2010

2.4 Biodynamic Preparations

There are eight preparations (Table 3); two of them are stirred in water following a special protocol and sprayed on plants and soil, the other six preparations are added to farmyard manure and all other organic materials produced on the farm. Modifications of this specific procedure have been developed, but in practice they are far less widespread than the original one, however in Elf the farmer adopted one of these (500P).

Spray preparations applied to soil and crops:	
500	Horn manure
501	Horn silica
Compost preparations used for preparing compost	
502	Yarrow blossoms (from Achillea millefolium)
503	Chamomile blossoms (from Matricaria chamomilla)
504	Stinging nettle (stalk from Urtica dioica)
505	Oak bark (Quercus robur)
506	Dantelion flowers (Taraxacum officinale)
507	Valerian (juice of flowers of Valeriana officinalis)

Table 3: Biodynamic spray preparations (500 - 501) and compost preparations (502 to 508).

In this study we consider the effect of the spray preparation alone for stimulating the fertility of the soil and general plant wellness.

500: (*horn-manure*) a humus mixture prepared by filling the horn of a cow with cow manure and burying it in the ground (40–60 cm below the surface) in the autumn. The number of treatments is depending on the area; but usually 2-3 times per year. During spring (end of March beginning of May) and autumn (from 15th August to beginning November) before soil became too cold. The application of the horn-manure should be in the late afternoon with no rainy weather condition and not too hot (Masson 2011).
501: (*horn silica*) crushed powdered quartz prepared by stuffing it into a horn of a cow and buried into the ground in spring and taken out in autumn. The treatment consists in a mixture of 1 tablespoon of quartz powder to 250 litres of water. The mixture is sprayed under very low pressure over the crop during the wet season, in an attempt to prevent fungal diseases and better perception of light for photosynthesis. It should be sprayed on an overcast day or early in the morning to prevent the burning of the leaves.
In the biodynamic eco-system farming the use of all eight treatments is important, for a better performance of the 500 and 501 treatments object of this study.

2.4.1 Dynamisation

100 gr./He of 500 are released a tank with 40-60 litres of water (preferably rain water) at 37 °C, while 501 about one teaspoon (4 gr./He) this solution is dynamised for an hour, by hand, or with an electric device starting a vortex in alternate directions.
The vortex created by stirring in one direction is created very fast and immediately broken stirring the fluid in the contrary direction. An explanation for this procedure (500) is that the creation of a vortex (oxygenation of the water) at this temperature increases the number of microorganisms in the solution.

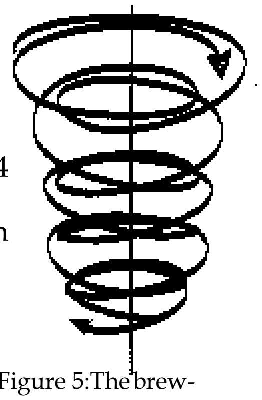

Figure 5:The brewing process: forming a vortex just before reversing the direction of rotation, start brewing in the opposite direction, chaos, forming a new funnel.

2.5 Soil

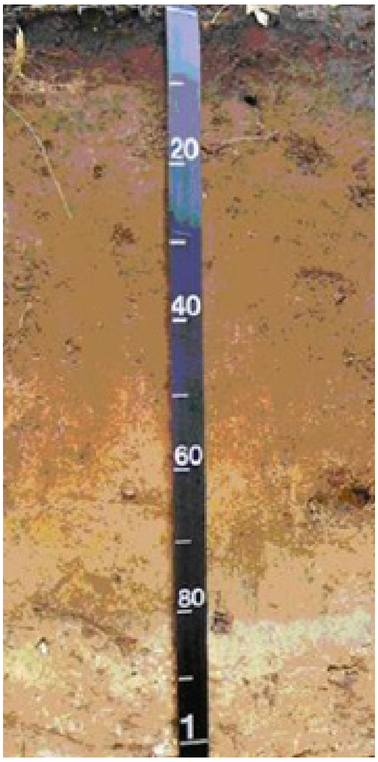

Figure 6: FAO classification soil: Cambisol.

2.5. Soil description

The soil in Haut is a carbonated soil with a rocky surface on glacial sand bed with a strong encrusting trend. Auv shows a heavy deep soil, free of stones, where roots reach deep into the ground. Ech has calcareous soil with an average texture on moraine-molasses compact soil bed of 55 cm with presence of calcareous accumulation (Letessier 2001). For FAO (World reference base) classification these three sites are Be (Eutric Cambisol) In Elf, the soil has a lot of clay and calcium, compacted and with a high pH.(Vertic Cambisol).

2.5.2 Standard soil chemical analyses

In June 2008 and at the end of the experiment in 2013, soil at 0 to 25 cm from both near the vines and between the rows samples from all four plots taken, were analyzed for the presence of mineral nutrients. The soil samples from each of the plots, 7(from the strip) + 7(from the middle) where united into a single sample of soil to be analysed. The samples were sieved at FiBL and pre-dried at 105 °C, after that they were sent to a specialized laboratory for analysis (Schweizer, Thun). The nutrient contents were measured with a solution volume 1: 10 of NH4 acetate extract for the "reserve" content (marked for eg. P) and H2O extract (marked for eg. Pw) for content easily available to plants.

The method AAE10 ex-lbu corresponds to the reference method AAE10 research institutions Agroscope. (J.-L. Spring et al. 2003) the results are expressed in mg/kg dry matter.

	correction factors of the standard fertilizer									
	1.5	1.4	1.3	1.2	1.1	1	0.9	0.8	0.0	0.0
	appreciation of the richness of the soil									
	Poor A	Mediocre B		Satisfactory C					Rich D	Really rich E
Element	Lightweight soils: less than 10% clay									
P	<40	40	60	80	85	90	100	110	120	>=240
K	<30	30	45	60	70	80	100	110	120	>=240
Mg	<50	50	75	100	120	140	160	180	200	>=400
Element	Means soil: 10 to 29.9% clay									
P	<20	20	30	40	45	50	60	70	80	>=160
K	<60	60	90	120	130	140	160	180	200	>=400
Mg	<75	75	110	150	175	200	225	250	300	>=600
Element	Heavy soils: 30% or more clay									
P	<10	10	15	20	23	25	30	35	40	>=80
K	<90	90	135	180	200	220	260	280	300	>=600
Mg	<100	100	150	200	230	260	300	350	400	>=800

Figure 7: Viticulture Soil values (Données de base pour la fumure en viticulture)

The weight fractions of clay and the silt size fractions of a soil sample aliquot are determined using samples from sedimentation in aqueous suspension. (Données de base pour la fumure en viticulture, J. Spring et al. 2003)

2.5.2. Organic Carbon and Humus

The organic carbon in the soil (Corg) is determined by a potassium-sulphuric acid extraction according to the formula:

2 Cr (VI) $2O7\ 2-$ + 16 $H+$ + 3 C(0) = 4 $Cr3+$ + 8 $H2O$ + 3 $CO2$ (Sims and Wolf 1995)

Humus is the total dead organic matter of a soil. The standard conversion factor from tot Corg to humus is 1.725 (Sims and Wolf 1995).

2.5.3 Soil microbial activity

In 2009 at FiBL the microbial biomass and their activity was monitored. Like Vance (1987) and Brookes (1985) the chloroform fumigations-extractions method was employed to analyze soil samples from all the farms and trial plots. Chloroform fumigation-extraction was done on 20 g (dry matter) subsamples that were extracted with 80 ml of a 0.5-M K2SO4 solution. The filtered extracts were analyzed for soluble C and N in a TOC-TNb analyzer.

Soil Cmic and Nmic were determined according to the following formulas:

Cmic=EC/kEC

Where EC=(TOC in fumigated samples-TOC in unfumigated control samples) and kEC=0.45 (Joergensen 1996).

Nmic =EN/kEN

Where EN=(Nt in fumigated samples- Nt in non fumigated control samples) and kEC=0.54 (Joergensen and Mueller 1996)

results are expressed in mg/Cmic/kg dry matter and mg/Nmic/kg dry matter and the ratio Cmic/Nmic.

2.5.4 Aggregate size distribution and quality

Aggregate size distribution and quality was done in 2009 and 2011. For the soil evaluation, the semi-quantitative method of Agroscope, or FAL (Nievergelt et al 2002) was used. The visual evaluation of soil structure starts with a falling test carried out with a soil sample. This falling test from 1m allows for breaking up the whole compact soil structure in single structural units. Afterwards the structural units are separated in size classes (10cm, 5 cm,2 cm, 1 cm, 0.5 cm, 2mm and 0.2 mm) using a battery of sieves, and the resulting size-fractions are weighed. In these size-fractions the occurring structural unit types are characterized by their geometrical form (contour, length of axes, angles and edges) and their surface qualities (roughness and visible pores). In addition the resistance of the structural unit types against mechanical stresses is tested and the fraction of the smallest structural units elements (< 0. 2mm) determined.

1- Taking sample and identification and separation of layers
2- Drop test (for observation cohesion of the structure on)
3- Sieving (identification the categories of sizes)
4- Weighing (pondered part)
5- Identification (type of structural units)
6- Test pressure between two fingers (cohesion of structural units)
7- Structural units notes and corrections (quality of the structure by size category)
8- Numerical evaluation of the structure (quality of the layer structure)

Figure 8: According to FAL-method fractionating a spade trial by seven aggregate large classes

The earth per fraction is removed, weighed and the % share is calculated on the total weight; then the units are described (e.g. crumbs, friability, polyhedra) and counted.

The results of this procedure are evaluated using a point system which is partly quantitative and partly semi-quantitative; the structure of every analysed soil layer is numerically expressed by weighted average of points.

From visual analyses it was possible to recognize some difference in the structure between the plots. For this reason it was decided to adopt this method of evaluation.

2.6 Plant analysis

2.6.1 Nutrients concentration and chlorophyll index of leaves

Figure 9:Washing leaves in distilled water with 4 g/L citric acid then dried and sent to the special laboratory.

In June, July and August 2009-2010, 25 leaves per plot (1 per plant) samples were collected in each plot from the fruit cane area region for the analysis of the nutriment concentration. In addition from the same leaves the chlorophyll index was measured with a hand-hold device “SPAD chlorophyll index” 502 Plus, Konika Minolta (Japan). At FiBL, the leaves were washed with citric acid 20gr X 5L 0.004 M and cleaned in distillate water, then dried at 65 °C for 12 h and at 105 °C for other 12 hours and sent to a specialized laboratory (Zeeuws-vlaanderen, Netherland) for analysis, where their contents of N, P, K, Ca, Mg, Mn, Cu, Fe, B were determined by spectroscopic techniques.

2.6.2 Plant growth

In 2009 and 2010, plant growth was measured on all sites and plots by taking the weight (kg m 2-1) of the shoots pruned in winter (wood weight), and in 2008 the length (cm) was measured as well.

Figure 10: Measuring with hand-hold device "SPAD chlorophyll index" 502 Plus, Konika Minolta (Japan) always in the same area of the leaf.

2.7 Disease management

Summary of periods of treatment and type of product employed for the control of the main diseases and pests.

Treatment period (according to Baggiolini and decimal code BBCH)	**Disease / Pest**	**Products (dosage in kg / ha)**
In cotton bud (B, 01) until green tip) Green tip	Erinose, Acariose Excorios	Sulfur (10–15) Sulfur (16)
Leaves come out leaves unfolded	Vers de la grappe Oidium, Excoriose Rougeot Oidium	Confusions techniques Sulfur (5) * Myco-San (4–16) + event. sulfur* (1–2)
Separate cluster	Mildew and Oidium, Rougeot	Myco-San (5–9) + event. sulfur* (1–2) *or* Myco-Sin (4–7) + sulfur (3–4)
Flower Buds	Mildew and Oidium, Rougeot	Myco-San (6–10) + event. sulfur* (1–2) *or* Myco-Sin (5–8) + sulfur (3–5) *or* copper (0.4–0.6) + sulfur (3–5)
Nouaison	Mildew and Oidium, Rougeot	Copper (0.6–0.8) + sulfur (3–5) *or* Myco-san (7–10) + event. sulfur* (1–2) *or* Myco-Sin (6–8) + sulfur (3–5)
Setting to closed cluster	Mildew and Oidium 2. generation cluster	Myco-San (7–10) + event. sulfur* (1–2) *or* Myco-Sin (6–8) + sulfur (3–5) Bacillus thuringiensis + sugar (10–15)
Closed early cluster	Mildew and Oidium 2.generation cluster	Copper (0.6–0.8) + Pandorra (5) *or* Bio-Blatt (2.5) *or* sulfur (2–4) Myco-San (7–10) *or* Myco-Sin (6–8) + sulfur (2–3) Bacillus thuringiensis + sugar (15)
Closed cluster Véraison	Mildew et Oidium Dessèchement de la rafle**	Copper (0.6–0.8) + event. Pandorra (5) *ou* Bio Blatt (2.5) *ou* sulfur (2–3) Myco-sin (6–8) + sulfur (0–3) *ou* Myco-san (7–10) Magnesium sulphate ** (at 10% and 50% of the ripening: 18-20 kg per ha)

Table 4:Treatment protocol for disease management in organic viticulture edited by FiBL for organic farmers.

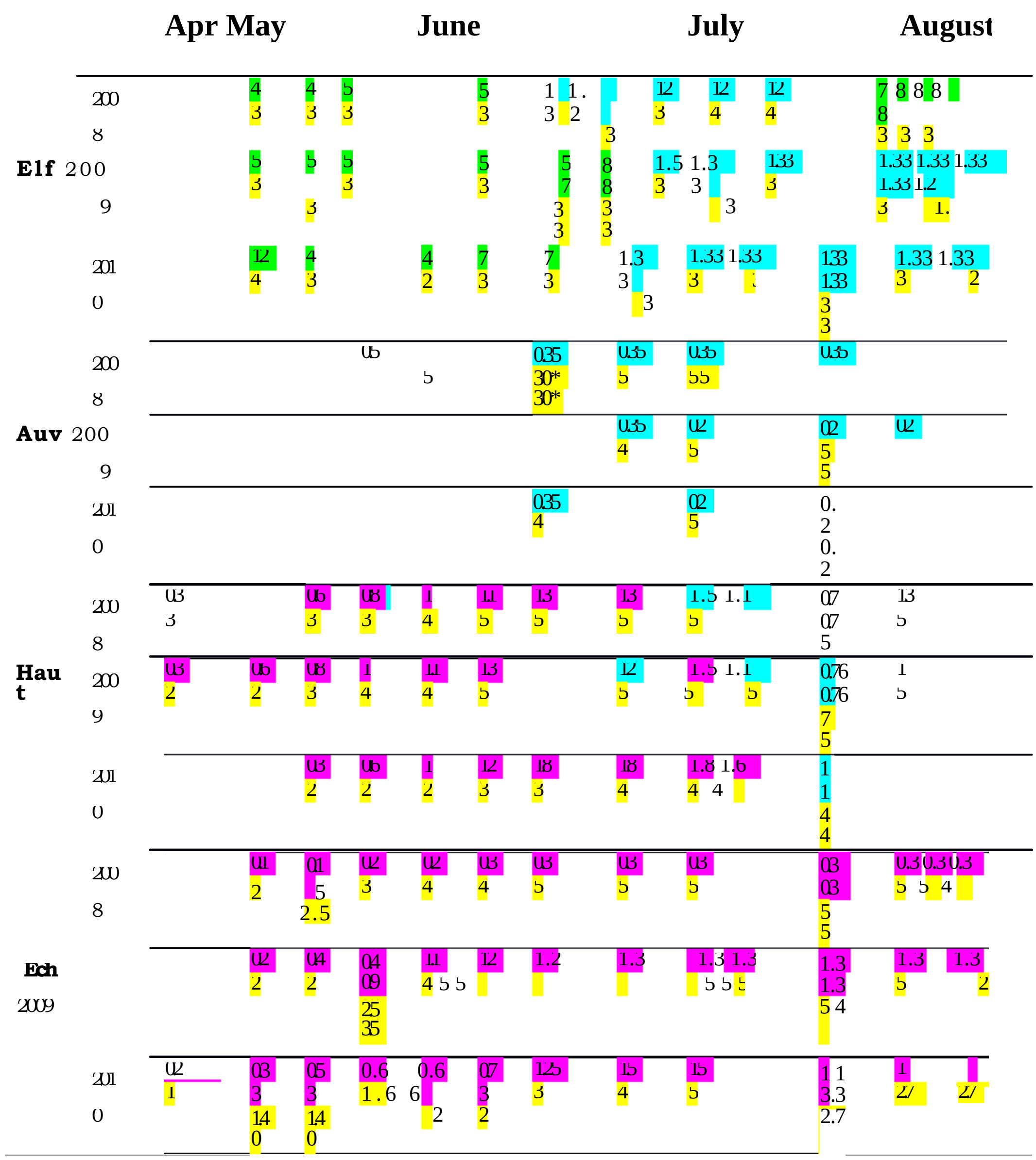

Table 5: Period of treatment of fungicides express in kg/he specially: Hydroxide Copper (Kocide Opti 30%), bordelaise mixture (20%), Sulfur (80% S) and clay-aluminum Myco-sin (65% S) from 2008-2010 for all sites.

Legend

C: Copper kg/ha S: Sulfur Wet sulfur kg/he S* Powder sulfur kg/he Clay-aluminum Sulfur Bordelaise mixture

2.7.1 Disease incidence and severity

In August and September 2008-2011 directly in the field, for each site and plot were observed 140 leaves per plant on 10 plants for a total of 1400 leaves, to see the incidence (proportion of leaves with symptoms) and the severity (proportion of diseased leaf area) and level of the Downy Mildew (Plasmopara viticola) and *Oidium (Oidium tuckeri).*

The number of leaves in each plot affected with the disease was recorded, and the percentage of incidence was calculated (dividing number of leaves affected by total number of leaves, and multiplying by 100), the severity level was observed according to the guideline used in FiBL laboratory in figure 10 (average of values from all leaves), and then the total severity was calculated by multiplying the percentage incidence by the severity level and then dividing the result by 100.

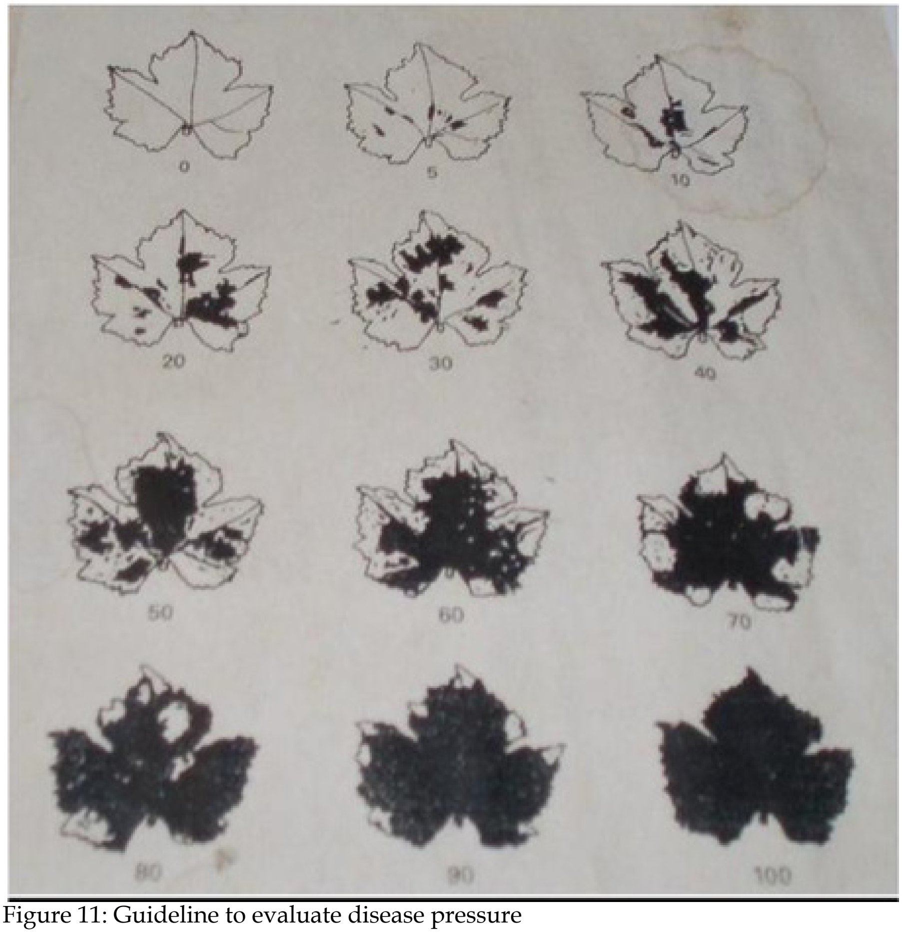

Figure 11: Guideline to evaluate disease pressure

2.7.2 Concentration of phytoalexins in leaves

The phytoalexins of vine leaves are indicators of the immunosystem and wellness of the plant. Phytoalexins (gr:phyto=:plant, alexins = to ward off) are low molecular weight antimicrobial chemical compounds that are immediately produced by the plant after being infected by microorganisms (such as bacteria or fungi), they act as toxins to the attacking organisms. In this study final analysis indicated as SumPhyto represents the sum of the main molecular components: stilbene, delta vyniferin and gamma vyniferin, resveratrol (stilbenoid, a type of natural phenol, and a phytoalexin produced naturally by several plants), piceide and phytoalexins. (R. Pezet et K. Gindro 2003).

For the analysis, in the Combi and Control plots, twenty leaves where collected per plant fruit cane area and then sent to the University of Neuchatel for processing at the ACW Changins-Nyon lab care of K. Gindor.

For the synthetic extraction of the phytoalexins separation was performed according to (R. Pezet et al 10) on a Dionex HPLC quaternary. The separation column was a column: Merck LiChrospher RP18 5 microns. The leaf fragments (max 8 mg) were placed in 50 microlitres of methanol and incubated for 10 min at 60 ° C under agitation. The supernatant used was directly injected, and as separation conditions Solvent A: H2O / Solvent B: acetonitrile were employed.

2.8 Grapes & Wine

2.8.1 Yield

In 2009 and 2010 at harvest, the weight in kg/vine for the Combi and Control plots were measured in each site.

2.8.2 Must analyses

In 2008-2010 the grapes for all four sites from batches Combi and Control were processed in a cluster pressing. A pneumatic press Sutter EPC -12, with a basket volume of 1200 L was used for processing the grapes. The juice was then fermented in a 25 L glass container.

From the test locations two variations were vinified in two replications based on the log of the previous years with the addition of the yeast strain ' Wädenswil W - 15'.

In 2008-2010 in the FiBL cellar the must analyses carried out with the standard method were: density / degree Oechsle (refract metric method), pH (pH-meter) and total acidity (neutralization with soda solution).

2.8.3 Wine analytic analyses

In 2008-2010 analytic analyses were performed for the wine from the Combi and Control plots by ACW, Changins Wadenswill Labor. Here were measured: alcohol [%vol.], density / degree Oechsle, fructose, glucose, total acidity, acetic acid, pH, glycerol, sucrose, lactic acid, volatile acidity, malic acid, tartaric acid all expressed in g/l.

2.8.4 Triangle test: Recognizing biodynamic wine

In March, 8th and 11th 2010 and May 2011 two triangle test the (sensorial testing) were carried out at the High school of Oenology in Changins by a panel of wine experts and students.

2.8.5 Sensorial test

In 2011 at FiBL several organoleptic tests were conducted separately with thirty-nine oenologists using 9 criteria: (Nose: fruity, green notes, minerality. Mouth: acid, green notes, fruitiness, body, texture, overall impression (general harmony)). Wines were evaluated and the rating written as a line in a 0-100 scale.

2.9 Statistical analysis

The data obtained in the field trials were analysed with ANOVA procedures using the software IBM SPSS Statistics (Statistical Package for the Social Sciences)". Where complete data sets were available from all sites and plots, a factorial model was used including site (AUV, ECH, ELF, HAU), biodynamic-preparation 500 (yes/no), biodynamic-preparation 501 (yes/no), and interactions 500*501, Site*500, Site*501. Case-wise (e.g. when we had data only from the Control and Combi plots, as e.g. with micro-vinified wines), we applied a simple model using site and treatment as the analysis of interactions is not possible in this case.
For the single sites analyses (Cmic, SPAD, wood weight, N, P) were carried out without considering the site Auv as this site had no 501 plots.

2.10 Holistic methods

These holistic methods would reveal information on the quality of the sample, and more specifically on its sanitary conditions, vitality, energy or degree of organization, among others. Given the influence that biodynamic agriculture is claimed to have on the quality of food, these analysis techniques could be more appropriate than conventional analysis for detecting the influence of biodynamic preparations.

2.10.1 Sensitive crystallization

Pfeiffer et Sabarth (1924) used a horizontal glass plate on which they crystallized a cupric chloride solution under temperature and hygrometry constants.
They noticed that the free crystallization of copper salt on a plate produced fine crystalline needles arranged typically without any order.
By adding infinitesimal amount of juice from a plant, or aqueous extracts of vegetable matter, the crystals of the sample tested were organized according to specific and reproducible patterns, in any case we are here considering an holistic analysis involving small quantities: trying to recognize a pattern of beams and branched needles radiate towards the periphery of the plate so as to reveal a

spectrum limit with discernible characteristics.

The objective of sensitive crystallization is not to reach a conclusion deduced from few isolated data, but to compare two established series of global phenomena and their order (A. et O. Selawry, 1957,1961).

2.10.2 Crystallization test on wine

In 2011 samples from wines 2008-2011 were sent at Laboratoire Thiollet- Margarethe Chapelle,Le Bourg 46220 Pescaidores France. The crystallization analyses the vitality, energy use and quality at stage 1 and a second judgement after 24h. As official scientific research does not accept this type of testing I have decided to include these methods in my thesis to point that there are also other testing methods which deliver interesting results which can be an aid in the understanding of the phenomena subject to our studies.

3 Results and Discussion

3.1 Soil

3.1.1 Standard soil chemical analyses: 2008 - 2013

The differences in soil composition from the beginning of the study in 2008 and at the end in 2013 only for plots Combi and Control for the four sites.

Dependent Variable : P

Source	Sum of squares	df	Mean Square	F	Sig.
Year	273,903	1	273,903	0,323	n.s.
Site	24687,385	3	8229,128	4,349	n.s.
Treat	1263,803	1	1263,803	0,536	n.s.
Treat* Year	846,81	1	846,81	2,228	n.s.
Treat * Site	5676,963	3	1892,321	4,979	*
Error	2280,547	6	380,091	0	

Table 6:Analysis of variance (ANOVA) for variable P the statistical differences of means are significantfor p< .05 (*) F significant a 5% and p< .01(**) F sig at 1%Figure 1: Bar graph showing the interaction between Treat and Site for the variable P

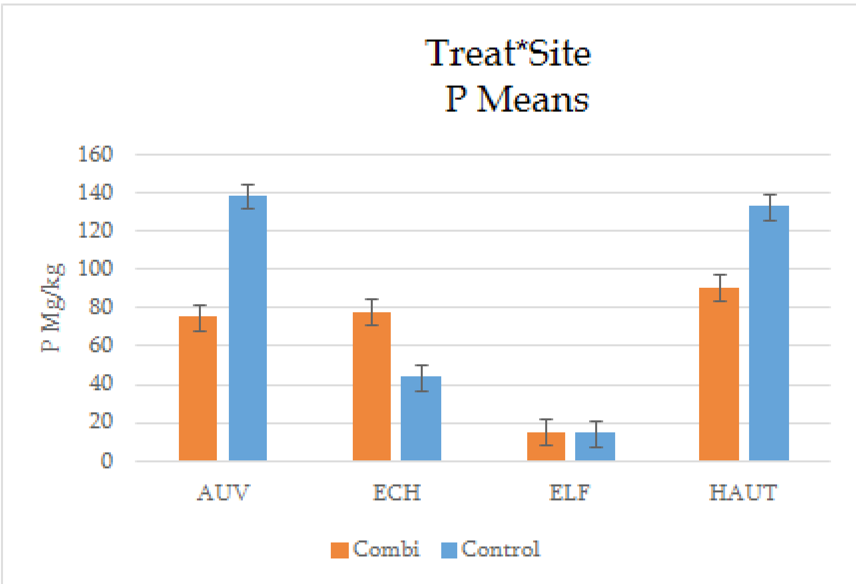

Figure 12:Bar graph showing the interaction between Treat and Site for the variable P.

P. The effect of the treatment is statistically significant just for the interaction Treat*Site. The graph shows a higher percentage in Combi plots only for Ech while the lowest in Haut and Auv (same region). The values of Elf remained stable. These differences in the value of P are ascribable to the differences of the soil, however the Combi treatment seems to have reduced the quantity.

Dependent Variable: K					
Source	Sum of squares	df	Mean Square	F	Sig.
Year	8145,063	1	8145,063	5,369	n.s.
Site	145345,82	3	48448,607	51,109	**
Treat	30835,36	1	30835,36	8,57	*
Treat* Year	1517,103	1	1517,103	0,422	n.s.
Treat * Site	2843,85	3	947,95	0,264	n.s.
Error	21569,235	6	3594,872	0	

Table 7:Analysis of variance (ANOVA) for variable K the statistical differences of means are significant for p< .05 (*)F significant a 5% and p< .01(**) F sig at 1%Figure 2: Bar graph showing the means of treatment for the variable K

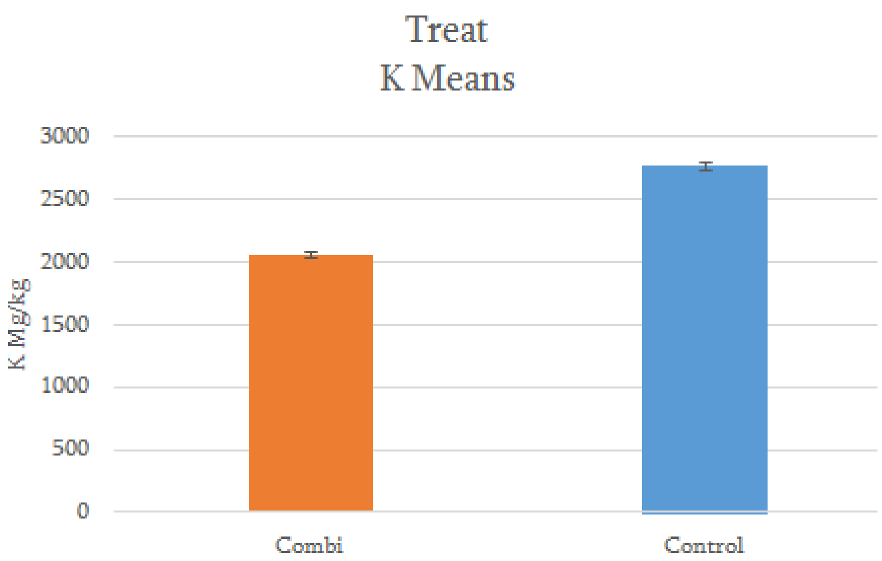

Figure 13:Bar graph showing the means of treatment for the variable K

K. The treatment effect is highly significant for site and at 5% for treatment effect. In the graph Control plots show a higher level of “reserve” potassium. This decrease of an important macroelement during the study period could be connected with the exhausting of the soil from the microorganisms, as we shall examine later.

Dependent Variable: Ca					
Source	Sum of squares	df	Mean Square	F	Sig.
Year	59138407	1	59138407,022	48,154	n.s.
Site	1290913711	3	430304570,18	2,224	n.s.
Treat	112080334	1	112080334,24	0,685	n.s.
Treat* Year	1228107,24	1	1228107,24	0,04	n.s.
Treat * Site	580381574	3	193460524,55	6,234	*
Error	186185891	6	31030981,781є	0	

Table 8:Analysis of variance (ANOVA) for variable Ca the statistical differences of means are significant for p< .05(*) F significant a 5% and p< .01(**) F sig at 1%Figure 3: Bar graph showing the interaction between Treat and Site for the variableCa

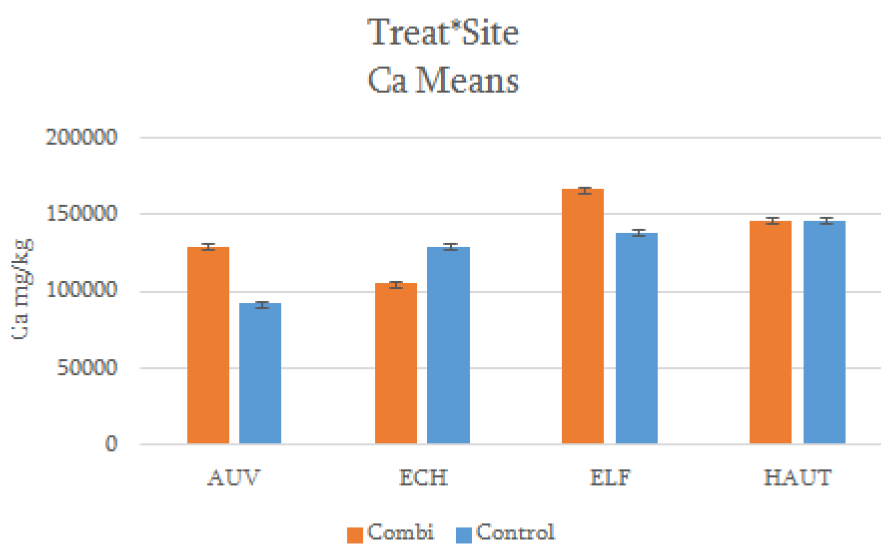

Figure 14: Bar graph showing the interaction between Treat and Site for variable Ca

Ca. Statistical significance for Ca level is shown in the interaction Treat*Site and only in Ech the level was lower in the Combi plot, in Haut remained the same. The presence of Ca is intimately connected with the type of soil and thus is difficult to interpret.

Dependent Variable: Mg					
Source	Sum of squares	df	Mean Square	F	Sig.
Year	20714,406	1	20714,406	155,952	n.s.
Site	928587,457	3	309529,152	8,401	n.s.
Treat	1511,266	1	1511,266	0,047	n.s.
Treat* Year	132,826	1	132,826	0,029	n.s.
Treat * Site	110536,047	3	36845,349	7,979	*
Error	27706,924	6	4617,821	0	

Table 9:Analysis of variance (ANOVA) for variable Mg the statistical differences of means are significant for p< .05(*) F significant a 5% and p< .01(**) F sig at 1%

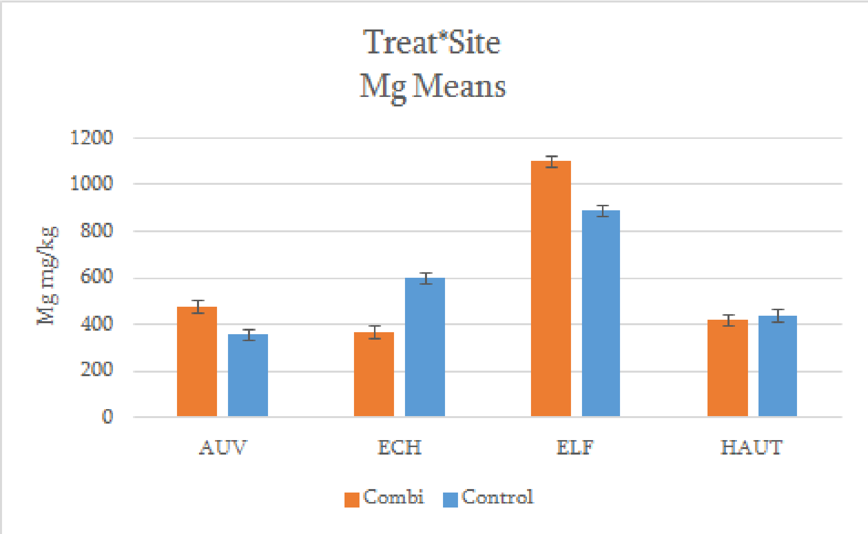

Figure 15:Bar graph showing the interaction between Treat and Site for the variable Mg

Mg. Also this depends to a large extent on the conditions of the soil, increasing for the Combi plots in Elf and Auv.

Dependent Variable: P w

Source	Sum of squares	df	Mean Square	F	Sig.
Year	1,103	1	1,103	0,136	n.s.
Site	178,853	3	59,618	8,218	n.s.
Treat	12,603	1	12,603	1,193	n.s.
Treat* Year	8,123	1	8,123	1,687	n.s.
Treat * Site	21,763	3	7,254	1,506	n.s.
Error	28,895	6	4,816	0	

Table 10:Analysis of variance (ANOVA) for variable Pw the statistical differences of means are significant for p< .05(*) F significant a 5% and p< .01(**) F sig at 1%

Dependent Variable: K w

Source	Sum of squares	df	Mean Square	F	Sig.
Year	235,622	1	235,622	1,237	n.s.
Site	3057,327	3	1019,109	6,242	n.s.
Treat	1115,56	1	1115,56	1128,253	n.s.
Treat* Year	190,44	1	190,44	0,54	n.s.
Treat * Site	489,8	3	163,267	0,463	n.s.
Error	2116,307	6	352,718	0	

Table 11:Analysis of variance (ANOVA) for variable Kw the statistical differences of means are significantfor p< .05 (*) F significant a 5% and p< .01(**) F sig at 1%

Dependent Variable: Ca w

Source	Sum of squares	df	Mean Square	F	Sig.
Year	4182,856	1	4182,856	41,62	n.s.
Site	19025,197	3	6341,732	111,908	**
Treat	253,606	1	253,606	0,6589	n.s.
Treat* Year	100,501	1	100,501	0,261	n.s.
Treat * Site	170,007	3	56,669	0,147	n.s.
Error	2309,299	6	384,883	0	

Table 12:Analysis of variance (ANOVA) for variable Caw the statistical differences of means are significantfor p< .05 (*) F significant a 5% and p< .01(**) F sig at 1%

Dependent Variable: Mg w

Source	Sum of squares	df	Mean Square	F	Sig.
Year	8,556	1	8,556	81	n.s.
Site	9,182	3	3,061	0,745	n.s.
Treat	8,556	1	8,556	2,007	n.s.
Treat* Year	0,106	1	0,106	0,025	n.s.
Treat * Site	12,317	3	4,106	0,964	n.s.
Error	25,564	6	4,261	0	

Table 13:Analysis of variance (ANOVA) for variable Mgw the statistical differences of means are significantfor p< .05 (*) F significant a 5% and p< .01(**) F sig at 1%

Dependent Variable: Humus					
Source	Sum of squares	df	Mean Square	F	Sig.
Year	0,083	1	0,083	0,771	n.s.
Site	6,496	3	2,165	20,81	*
Treat	1,15	1	1,15	13,523	n.s.
Treat* Year	0,107	1	0,107	0,85	n.s.
Treat * Site	0,312	3	0,104	0,824	n.s.
Error	0,758	6	0,126	0	

Table 14:Analysis of variance (ANOVA) for variable Humus the statistical differences of means aresignificant for p< .05 (*) F significant a 5% and p< .01(**) F sig at 1%

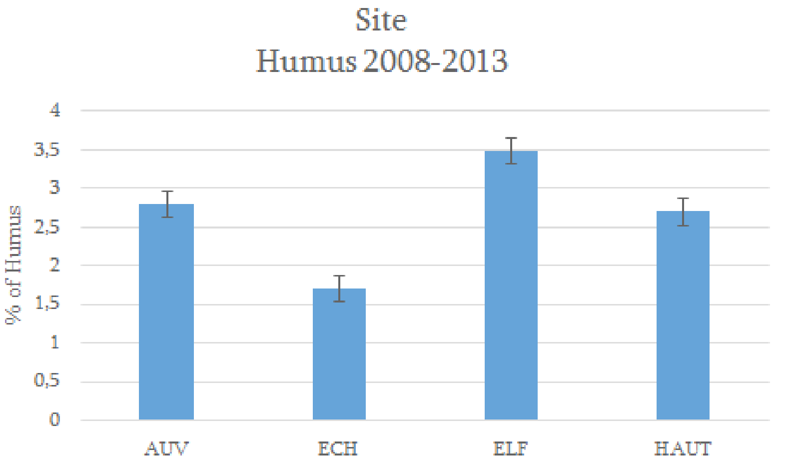

Figure 16: Bar graph showing the percentage of humus in single sites.

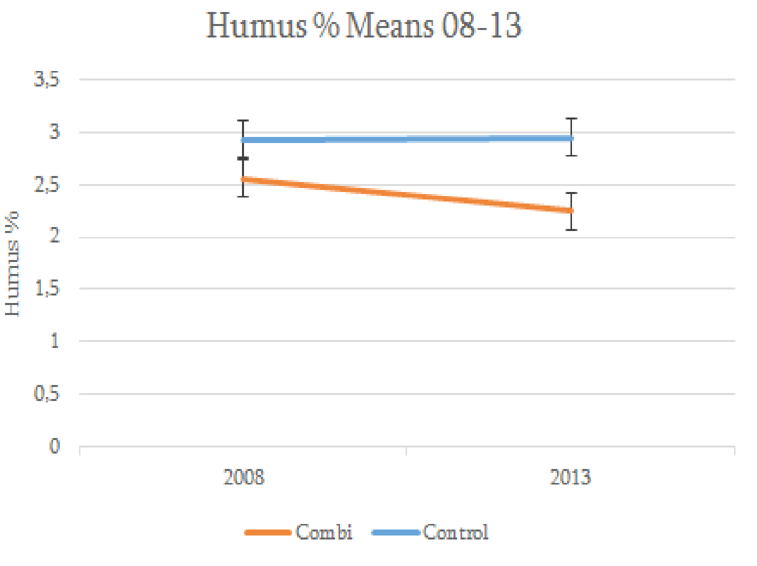

Figure 17: Graph showing the interaction between treatment and year forthe variable Humus. However this is statistically not significant but showthe trend of the humus percentage during the trial.

Humus. The Control plot in 6 years didn't show any variations only a minimum increase in humus percentage, on the contrary the Combi plots displayed a reduction in humus percentage. These graphs show that treatment 500 from the beginning of the study until the end decreased humus in 3 sites. In Ech from 1.62 % to 1.40 %, in Auv from 2.82 to 1.90 % (strong decrease) in Haut from 2.59 to 2.10%. Only in Elf the value increased from 3.20% to 3.60 %. In any case this is not significant, but it gives us an indication on what happened during the six years of the study. This trend when connected with the no manure supply strategy indicates that the clay soil of Elf after 500 treatment and manure supply preserved better the organic fraction in the soil.

3.1.2 Standard soil chemical analyses: Soil conditions in 2013

In 2013 chemical analyses with NH4 acetate and H2O extraction were carried and it was out and it was possible to effect analyses for single plots 500, 501, Combi and Control. In the tables, the ANOVA test of each element, when significant are shown.

Dependent Variable: P

Source	Sum of squares	df	Mean Square	F	Sig.
Site	19995,889	3	6665,296	12,692	n.s.
T500	2108,34	1	2108,34	2,979	n.s.
T501	350,938	1	350,938	0,77	n.s.
T500*T501	192,284	1	192,284	0,301	n.s.
T500*Site	2122,929	3	707,643	1,109	n.s.
T501 * Site	1367,488	3	455,829	0,714	n.s.
Error	1914,995	3	638,332	0	

Table 15: nalysis of variance (ANOVA) for variable P the statistical differences of means are significantfor p< .05 (*) F significant a 5% and p< .01(**) F sig at 1%

Dependent Variable: K

Source	Sum of squares	df	Mean Square	F	Sig.
Site	192941,347	3	64313,782	5,619	n.s.
T500	27683,414	1	27683,414	2,965	n.s.
T501	2320,028	1	2320,028	0,847	n.s.
T500*T501	2226,267	1	2226,267	3,54	n.s.
T500*Site	28009,521	3	9336,507	14,845	*
T501 * Site	8216,75	3	2738,917	4,355	n.s.
Error	1886,774	3	628,925	0	

Table 16:Analysis of variance (ANOVA) for variable K the statistical differences of means are significantfor p< .05 (*) F significant a 5% and p< .01(**) F sig at 1%

Figure 19:Bar graph showing the interaction between 500 and Site for the variable K

Dependent Variable: Ca

Source	Sum of squares	df	Mean Square	F	Sig.
Site	1205363383,333	3	401787794,444	4,805	n.s.
T500	9160711,111	1	9160711,111	0,343	n.s.
T501	75140002,778	1	75140002,778	0,785	n.s.
T500*T501	16469,444	1	16469,444	0	n.s.
T500*Site	80187483,333	3	26729161,111	0,688	n.s.
T501 * Site	287188791,667	3	95729597,222	2,465	n.s.
Error	116517658,333	3	38839219,444		

Table 17:Analysis of variance (ANOVA) for variable Ca the statistical differences of means are significant forp< .05 (*) F significant a 5% and p< .01(**) F sig at 1%

Dependent Variable: Mg

Source	Sum of squares	df	Mean Square	F	Sig.
Site	970747,23	3	323582,41	21,267	n.s.
T500	9320,293	1	9320,293	7,285	n.s.
T501	15349,145	1	15349,145	0,693	n.s.
T500*T501	6567,752	1	6567,752	0,801	n.s.
T500*Site	3837,89	3	1279,297	0,156	n.s.
T501 * Site	66417,422	3	22139,141	2,699	n.s.
Error	24609,562	3	8203,187		

Table 18:Analysis of variance (ANOVA) for variable Mg the statistical differences of means are significantfor p< .05 (*) F significant a 5% and p< .01(**) F sig at 1%

Dependent Variable: Pw

Source	Sum of squares	df	Mean Square	F	Sig.
Site	187,472	3	62,491	3,325	n.s.
T500	32,585	1	32,585	1,671	n.s.
T501	0,478	1	0,478	1,407	n.s.
T500*T501	3,642	1	3,642	3,468	n.s.
T500*Site	58,507	3	19,502	18,572	*
T501 * Site	1,02	3	0,34	0,324	n.s.
Error	3,15	3	1,05		

Table 19:Analysis of variance (ANOVA) for variable Pw the statistical differences of means are significantfor p< .05 (*) F significant a 5% and p< .01(**) F sig at 1%

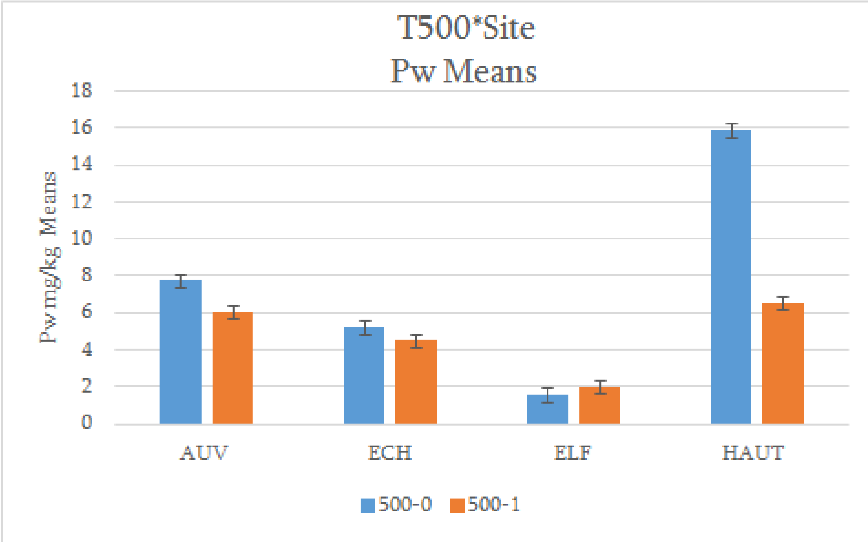

Figure 20:Bar graph showing the interaction between 500 and Site for the variable Pw.Means for Pw element available in water solution for the plants in four sites. Elfingenshows after treatment 500 a higher level of P

Dependent Variable: Kw

Source	Sum of squares	df	Mean Square	F	Sig.
Site	3775,556	3	1258,519	1,231	n.s.
T500	2316,818	1	2316,818	2,217	n.s.
T501	0,871	1	0,871	0,01	n.s.
T500*T501	387,434	1	387,434	3,446	n.s.
T500*Site	3135,765	3	1045,255	9,297	n.s.
T501 * Site	269,838	3	89,946	0,8	n.s.
Error	337,303	3	112,434		

Table 20:Analysis of variance (ANOVA) for variable Kw the statistical differences of means aresig-nificant for p< .05 (*) F significant a 5% and p< .01(**) F sig at 1%

Dependent Variable: Ca w

Source	Sum of squares	df	Mean Square	F	Sig.
Site	27048,225	3	9016,075	322,694	n.s.
T500	10,563	1	10,563	0,064	n.s.
T501	7,022	1	7,022	0,174	n.s.
T500*T501	262,44	1	262,44	1,481	n.s.
T500*Site	494,373	3	164,791	0,93	n.s.
T501 * Site	121,203	3	40,401	0,228	n.s.
Error	531,755	3	177,252		

Table 21:Analysis of variance (ANOVA) for variable Caw the statistical differences of means aresig-nificant for p< .05 (*) F significant a 5% and p< .01(**) F sig at 1%

Dependent Variable: Mg w

Source	Sum of squares	df	Mean Square	F	Sig.
Site	41,912	3	13,971	3,273	n.s.
T500	2,151	1	2,151	0,486	n.s.
T501	3,18	1	3,18	3,833	n.s.
T500*T501	2,3	1	2,3	2,322	n.s.
T500*Site	13,288	3	4,429	4,47	n.s.
T501 * Site	2,489	3	0,83	0,837	n.s.
Error	2,973	3	0,991		

Table 22:Analysis of variance (ANOVA) for variable Mgw the statistical differences of means are-significant for p< .05 (*) F significant a 5% and p< .01(**) F sig at 1%

Effect of preparation 500 and 501 on soil elements in 2013:

One notices a decrease in the level of the total soil element for the plot undergoing biodynamic treatments, though the significant result is just relative to K interaction T500*Site and Pw level in interaction T500*Site nearly significant (P=0.056). The application of the 500 treatment on all plots has shown evidence of a reduction in the soil of the "reserve" potassium and Pw. Only in Elfingen levels remain stable.

Could be that the plant adsorbed or it was dispersed following leaching, and the 500 preparation provided for a better adsorption.

The Kw total and soluble decreased after 500 treatment, this results suggest a higher availability of the element for the plant in the soil.

In a review Raupp and König (1996) show that biodynamic preparations cause the greatest effect under poor yielding conditions. An hypothesis is that biodynamic treatments have more influence in soil with worst characteristics. Revee et al. (2005) had the same results and is possible to attribute to the initial quality of the soil for chemical soil elements.

Dependent Variable: Humus

Source	Sum of squares	df	Mean Square	F	Sig.
Site	6,979	3	2,326	5,062	n.s.
T500	0,258	1	0,258	0,542	n.s.
T501	0,795	1	0,795	12,764	*
T500*T501	0,433	1	0,433	5,451	n.s.
T500*Site	1,43	3	0,477	5,996	n.s.
T501 * Site	0,187	3	0,062	0,783	n.s.
Error	0,239	3	0,08		

Table 23:Analysis of variance (ANOVA) for variable Humus the statistical differences ofmeans are significant for p< .05 (*) F significant a 5% and p< .01(**) F sig at 1%

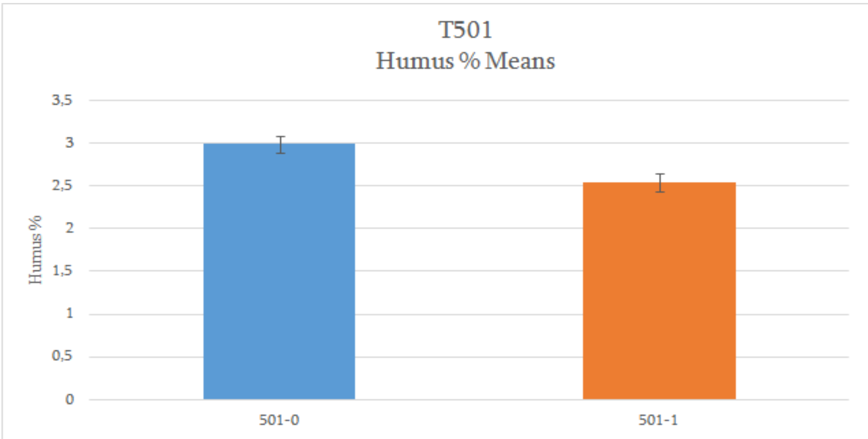

Figure 21:Bar graph showing the interaction between 500 and Site for the vari-ablehumus percentage.Treat*Site is not significant but nevertheless interesting for theextremely high level of humus in Elfingen after using treatment 500.

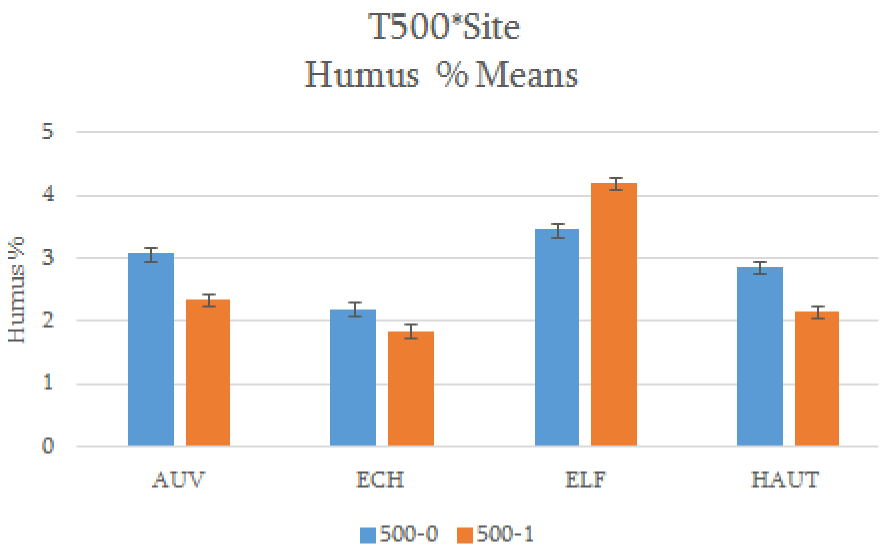

Figure 22:Bar graph showing the means of treatment 501 for the variable Humus-percentage. Significant influence of 501 in humus composition

3.1.2.1 Humus organic matter

The hypothesis is that this low content of humus is due to the fact that these soils were not fed, but only treated with 500 and 501 without the addition of green manure. What was the contribution in terms of organic substance? The rate of decomposition of the 500 preparation is quick, 500 is not a fertilizer but an activator of soil vitality. With 500 the transformers in the ground increase and consequently the humus present mineralizes an thus lowered the humus level. If organic substance is present humification process is activated, but if the quantity is low, it mineralizes immediately thus reducing humus quantity and the soil is poorer.

In general these are soils with 3% organic substance which is considered a high level, however it is exactly for this condition and climate trends with many mm of rain and spontaneous vegetation intercepting it. For these reasons reintegration with legumes for a supply of nitrogen, cereals for fibres for the soil structure and other essences which with their roots going deep bringing to the surface useful elements.

	P_{AAE10}	K_{AAE10}	Ca_{AAE10}	Mg_{AAE10}	P_w	K_W	Ca_w	Mg_w	Humus %
Auv	102.31	296.78	54912.50	408.21	7.00	55.62	182.75	7.73	2.78
Ech	69.88	187.70	53020.00	407.75	4.88	53.83	180.85	8.48	2.03
Elf	18.30	495.50	75597.50	991.28	1.80	49.40	275.88	12.05	3.83
Haut	114.90	350.10	68235.00	401.35	11.23	88.93	174.93	8.03	2.50
500/0	91.27	370.45	61245.00	561.43	7.68	72.57	202.81	9.24	2.93
500/1	61.43	294.59	64637.50	542.86	4.78	51.31	204.39	8.90	2.64
501/0	77.59	348.23	61713.75	536.03	6.38	63.11	205.86	9.71	2.99
501/1	75.10	316.82	64168.75	568.27	6.08	60.77	201.34	8.43	2.58
P-value		0.16						0.09	0.16
Interaction	--	Site*500 0.06	--	--	Site*500 0.07	Site*500 0.1	--	Site*500 0.1	Site*500 0.20 500*501 0.19

3.1.3 Soil microbial activity

Soil microbial activity results for site, treatment, position and depth are shown in the following tables.

Dependent Variable: Cmic

Source	Sum of squares	df	Mean Square	F	Sig.
Site	918050,594	3	306016,865	2,289	n.s.
Treat	1391,281	1	1391,281	0,819	n.s.
Position	5644,531	1	5644,531	0,366	n.s.
Deep	960151,531	1	960151,531	7,819	n.s.
Treat * Position	6022,531	1	6022,531	1,921	n.s.
Treat * Deep	94,531	1	94,531	0,03	n.s.
Position * Deep	1046,531	1	1046,531	0,334	n.s.
Treat * Site	5096,844	3	1698,948	0,542	n.s.
Position * Site	46321,594	3	15440,531	4,925	*
Deep * Site	368392,594	3	122797,531	39,17	**
Error	40754,406	13	3134,954		

Table 24:Graph showing the interaction between Site and Position for the variable Cmic.Shows microbial activity near the plant or in the middle of the strip. One can notice that forthree sites these values were higher for the middle of strip, and in Elfingen near the plants.Furthermore the activity shown in Elfingen is higher. Data expressed in mg Cmic/kg of drymatter.

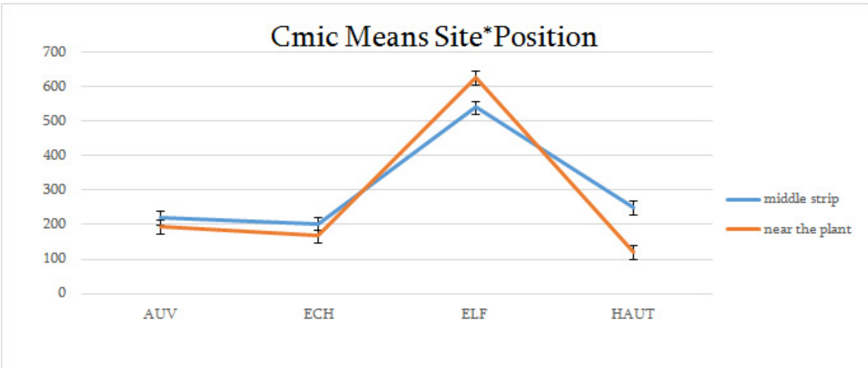

Figure 23:Graph showing the interaction between Site and Position for the variable Cmic.Shows microbial activity near the plant or in the middle of the strip. One can notice that forthree sites these values were higher for the middle of strip, and in Elfingen near the plants.Furthermore the activity shown in Elfingen is higher. Data expressed in mg Cmic/kg of drymatter.

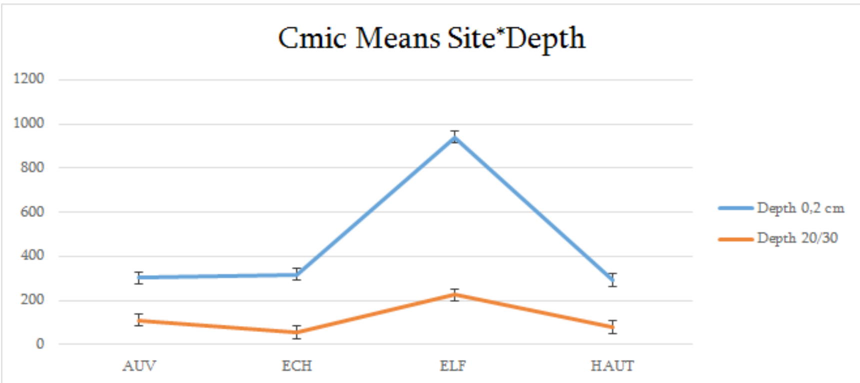

Figure 24:Graph showing the interaction between Site and Deep for the variableCmic. Highly significant is activity in surface stratum -0.2 cm than at a depth of20-30 cm. Elf shows higher activity in both strata. expressed in mg Cmic/kg of drymatter.

Cmic. Interaction Depth*Site it's highly significant. In all sites, the microbial activity was higher in the superficial part than in the 20/30 cm part. The treatment effect didn't show any effect, however we have to consider that in absence of a supply of manure the growth is seriously compromised and the values detected are low confirming the good status in Elfingen. Also the depth ANOVA result is at limit but the interaction Depth*Site is statistically significant.

The carbon of microorganisms shows higher values near the plant in the rhizosphere only in Elfingen, this could be correlated with the percentage of humus and its increase. In the other three sites it was higher in the inter row. This could be correlated with copper and sulphur treatments.

The effect of the treatment is not significant but displays a higher value in the Control plots rather than in the Combi plots, contrary to the literature.

Dependent Variable: N mic

Source	Sum of squares	df	Mean Squares	F	Sig.
Site	24558,594	3	8186,198	2,032	n.s.
Treat	19,531	1	19,531	0,369	n.s.
Position	457,531	1	457,531	0,968	n.s.
Deep	35178,781	1	35178,781	9,147	n.s
Treat * Position	569,531	1	569,531	3,326	n.s.
Treat * Deep	166,531	1	166,531	0,972	n.s.
Position * Deep	87,781	1	87,781	0,513	n.s.
Treat * Site	158,594	3	52,865	0,309	n.s.
Position * Site	1418,094	3	472,698	2,76	n.s.
Deep * Site	11538,344	3	3846,115	22,46	**
Error	2226,156	13	171,243		

Table 25:Analysis of variance (ANOVA) for variable Nmic the statistical differences of means are significant for p< .05 (*) F significant a 5% and p< .01(**) F sig at 1%

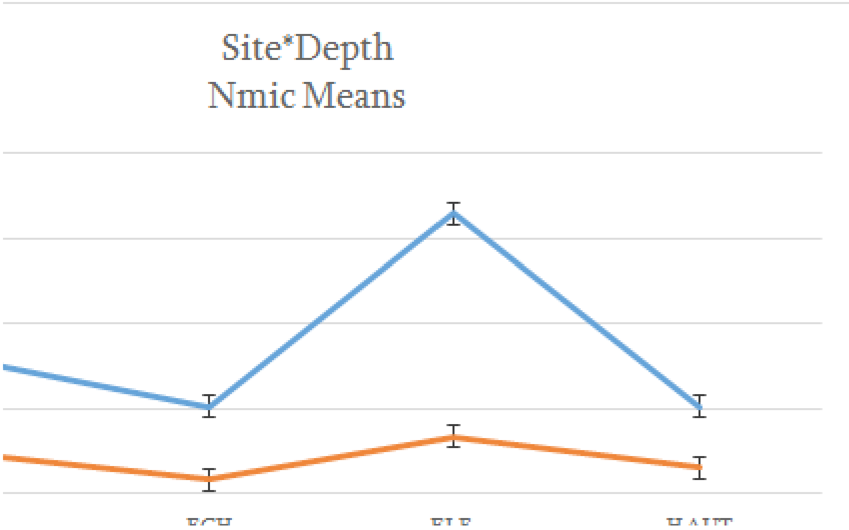

Figure 25:Graph showing the interaction between Site and Deep for the variable Nmic .Nmic results show the same trend as for Cmic. Elfingen also in this case has highervalues. expressed in mg Cmic/kg of dry matter.

Nmic. Treatment shows no effect for Nmic values. Depth*Site is strongly statistically significant with high differences in Elfingen.

Dependent Variable: C mic / Nmic

Source	Sum of squares	df	Mean Square	F	Sig.
Site	21,411	3	7,137	6,904	n.s.
Treat	1,026	1	1,026	4,29	n.s.
Position	0,326	1	0,326	2,098	n.s.
Deep	0,376	1	0,376	0,357	n.s.
Treat * Position	0,528	1	0,528	2,551	n.s.
Treat * Deep	0,049	1	0,049	0,236	n.s.
Position * Deep	0,167	1	0,167	0,806	n.s.
Treat * Site	0,717	3	0,239	1,156	n.s.
Position * Site	0,466	3	0,155	0,751	n.s.
Deep * Site	3,159	3	1,053	5,089	*
Error	2,69	13	0,207		

Table 26:Analysis of variance (ANOVA) for variable Cmic / Nmic the statistical differences of means are significantfor p< .05 (*) F significant a 5% and p< .01(**) F sig at 1%Figure 14: Bar graph showing the interaction between Site and Deep for thevariable Cmic/Nmic. Cmic/Nmic expressed in mg Cmic/kg of dry matter.

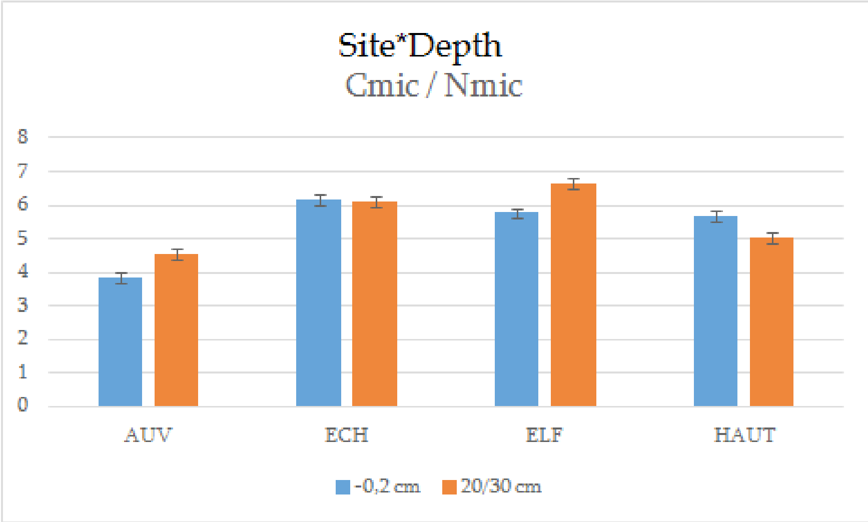

Figure 26:Bar graph showing the interaction between Site and Deep for thevari-able Cmic/Nmic. Cmic/Nmic expressed in mg Cmic/kg of dry matter.

3.1.3.1 Single site microbial statistical analysis

In order to gain more detailed results statistics were carried out for the single sites. Here following the values for Hauterive.

Dependent Variable: Cmic

Source	Sum of squares	df	Mean square	F	Sig.
Treat	1152	1	1152	144	*
Position	33024,5	1	33024,5	4128,062	**
Deep	92020,5	1	92020,5	11502,562	**
Treat * Positior	420,5	1	420,5	52,563	n.s.
Treat * Deep	924,5	1	924,5	115,563	n.s.
Position * Deep	15488	1	15488	1936	**
Error	8	1	8		

Table 27:Analysis of variance (ANOVA) for variable Cmic the statistical differences of means are significantfor p< .05 (*) F significant a 5% and p< .01(**) F sig at 1%

Dependent variable: N mic

Source	Sum of square	df	Mean square	F	Sig.
Treat	60,5	1	60,5	121	n.s.
Position	1058	1	1058	2116	*
Deep	2592	1	2592	5184	**
Treat * Positic	12,5	1	12,5	25	n.s.
Treat * Deep	24,5	1	24,5	49	n.s.
Position * Dee	512	1	512	1024	*
Error	0,5	1	0,5		

Table 28:Analysis of variance (ANOVA) for variable Nmic the statistical differences of means are significant for p< .05 (*) F significant a 5% and p< .01(**) F sig at 1%

Dependent Variable C mic / Nmic

Source	Sum of squares	df	Mean square	F	Sig.
Treat	0,092	1	0,092	1,048	n.s.
Position	0,076	1	0,076	0,862	n.s.
Deep	0,952	1	0,952	10,796	n.s.
Treat * Positi	0,029	1	0,029	0,327	n.s.
Treat * Deep	0,008	1	0,008	0,096	n.s.
Position * Dee	0,13	1	0,13	1,474	n.s.
Error	0,088	1	0,088		

Table 29:Analysis of variance (ANOVA) for variable Cmic / Nmic the statistical differences of means aresignificant for p< .05 (*) F significant a 5% and p< .01(**) F sig at 1%

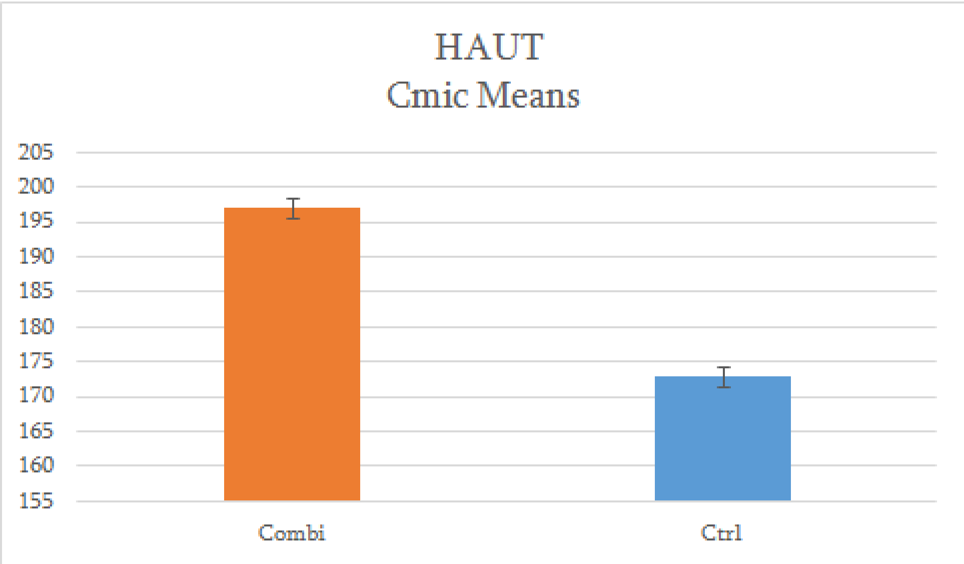

Figure 27:Bar graph showing the means of treatment for the variable Cmic inHaut. In Haut significant effect of treatment for Combi plots that showhighest activities. Cmic expressed in mg Cmic/kg of dry matter.

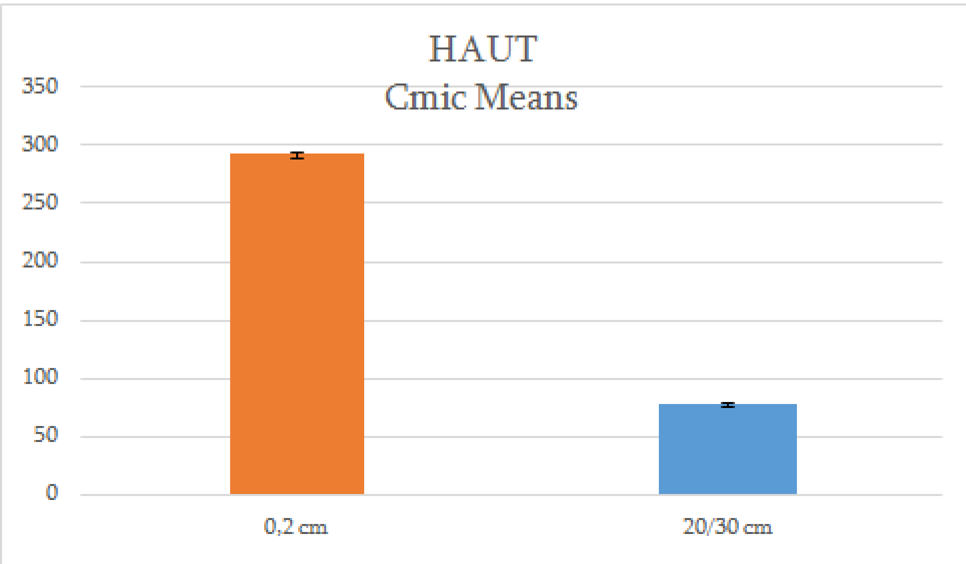

Figure 28:Bar graph showing the means of treatment for the variable Cmic inHaut .Cmic shows significant results for 0.2 cm activity expressed in mg Cmic/kg of dry matter.

Results:

In Haut the treatment effect is near the significant (p=0.053), strong depth and position. Combi parcel has results higher than the Control plots.

In the other three sites(data not shown) the Cmic value was higher in the Control parcel and the position 1 (middle of vineyard row) was higher than position 2 (near the plant trunk). Only in Elf the microorganism activity was higher in position 2, this trend could be connected with the Copper- Sulphur management. Depth results show a higher activity in the first 0.2 cm then the 20/30 cm.

3.1.4 Quality of the soil aggregate

In 2009 analysis of variance of the aggregate quality values shows that Combi plots with 27.00 weight average of points are significantly higher than the Control plots with 25.75. The most significant improvement is the quality of the aggregates in Elf, less - but still recognizable - in Ech and Haut. These results show also a statistical Site effect. Reganold in New Zealand described biodynamic soils with a better structure, aeration and consequently tilth (Reganold 1998); but the comparison system was a conventional trial and not organic.

After two years, in 2011, applying the same procedure, the results averaged over the different size classes of soil quality indices which were not replicated. For Auv and Ech, similar values for Control with Combi slightly higher (7.9% and 10.1%). In Ech the indexes was 7.85 for Control and 8.72 for Combi. In Elf almost no difference was measurable (Control 4.99, 4.89 Combi). All this data is not shown.

Across all sites the differences between the methods was not significant (P-value= 0.303), the effects of the sites, however, vary always from significant to highly significant.

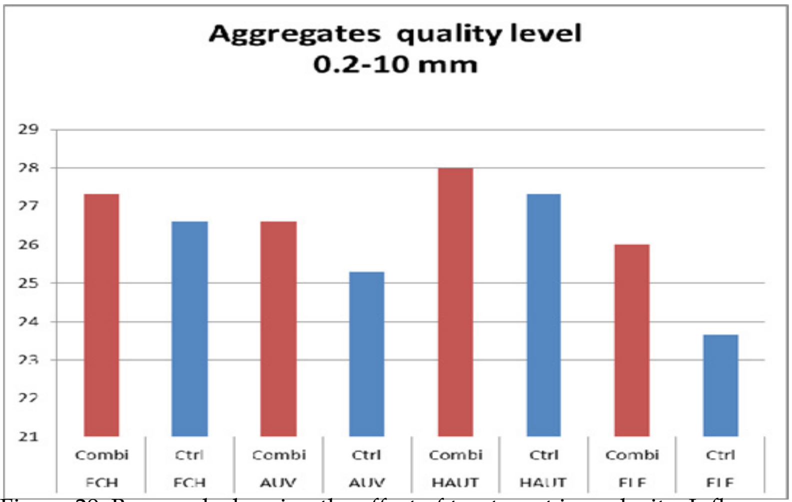

Figure 29: Bar graph showing the effect of treatment in each site. Influence of treatments in 2009 on the quality of numerical values of the soil aggregates of size 0.2-10mm (FAL method) expressed in weight average per point. Expressed inweighted average of points.

For the type of aggregates the most here could only be distinguished between two forms, whereas a more differentiated assessment was possible in the middle classes. An improvement in Auv and Ech and a deterioration in Haut with Combi. However, the index values for all indexes Combi are higher than those of the Control.

3.2 Plant results

3.2.1 Nutrient concentration in leaves

In 2009-2010 the evaluation of the leaf nutrient contents and values showed the following results in tables and when significant also in graphs. The results for N, P, SPAD and wood weight where such that the decision was taken to carry out a statistical analysis for each single site (data supplied at the end of the chapter):

Dependent Variable: N

Source	Sum of squares	df	Mean square	F	Sig.
Year (Y)	0,002	1	0,002	0,224	n.s.
Site (S)	0,025	2	0,013	0,409	n.s.
Block (B)	0,002	2	0,001	0,098	n.s.
Interaction YxS	0,032	2	0,016	1,491	n.s.
Treatment 500 (T500)	0,035	1	0,035	1,853	n.s.
Treatment 501 (T501)	0,037	1	0,037	4,202	n.s.
Interaction T500xT501	0,002	1	0,002	0,164	n.s.
Interaction YxT500	0,001	1	0,001	0,092	n.s.
Interaction YxT501	0,012	1	0,012	1,126	n.s.
Interaction SxT500	0,057	2	0,029	2,663	n.s.
Interaction SxT501	0,015	2	0,007	0,683	n.s.
Error	0,593	55	0,011	0	

Table 30:Analysis of variance (ANOVA) for variable N the statistical differences of means are-significant for p< .05 (*) F significant a 5% and p< .01(**) F sig at 1%

N. For all four sites no significant difference method showed on the leaf-N contents. Most striking was by far the lowest supply - at optimal levels between 1.93 to 2.31 % of dry matter (reference values from “Données de base pour la fumure viticulture”) - with values around 1.4 % of the dry matter on the farm in Auv. Also the statistical analysis in Ech shows no significant N-sheet content but the process with 501 with 2.03 % N of dry matter was higher than other methods (data not shown). This contradicts the Combi method showing 1.83% N of dry matter in the cases were also horn silica was applied.

In contrast, the method Haut Combi slightly exceeded (1.99%) and Control (1.93%).

In Elf all values were slightly below the limit of sufficient supply, but without differences between the methods.

Dependent Variable: P					
Source	Sum of squares	df	Mean square	F	Sig.
Year (Y)	0,051	1	0,051	3,2	n.s.
Site (S)	0,036	2	0,018	0,806	n.s.
Block (B)	0,000008	2	0,000004	0,047	n.s.
Interaction YxS	0,035	2	0,017	18,516	**
Treatment 500 (T500)	0,007	1	0,007	3,083	n.s.
Treatment 501 (T501)	0,006	1	0,006	1,911	n.s.
Interaction T500xT501	0,001	1	0,001	1,355	n.s.
Interaction YxT500	0,001	1	0,001	0,618	n.s.
Interaction YxT501	0,00001	1	0,00001	0,012	n.s.
Interaction SxT500	0,005	2	0,003	2,907	n.s.
Interaction SxT501	0,008	2	0,004	4,251	*
Error	0,051	55	0,001	0	

Table 31:Analysis of variance (ANOVA) for variable P the statistical differences of means are significant for p< .05 (*) F significant a 5% and p< .01(**) F sig at 1%

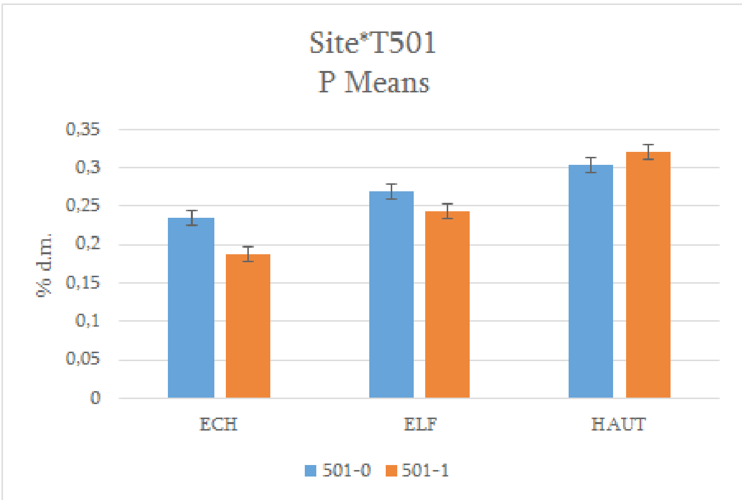

Figure 30:Bar graph showing the interaction between of 501 and Site for the variable P.Significant results after treatment 501 that show inferior quantities in P content of leavesfor Ech and Elf. Higher in Haut.

P. The phosphorus contents were inversely proportional as the N contents (this is called an "effect on the concentration") but for Phosphorus contents there were in turn mainly regional differences.

Dependent Variable: K					
Source	Sum of squares	df	Mean square	F	Sig.
Year (Y)	0,042	1	0,042	4,101	n.s.
Site (S)	6,934	2	3,467	34,294	n.s.
Block (B)	0,014	2	0,007	0,132	n.s.
Interaction YxS	0,095	2	0,047	0,907	n.s.
Treatment 500 (T500)	0,165	1	0,165	1,781	n.s.
Treatment 501 (T501)	0,003	1	0,003	0,117	n.s.
Interaction T500xT501	0,34	1	0,34	6,524	*
Interaction YxT500	0,001	1	0,001	0,014	n.s.
Interaction YxT501	0,066	1	0,066	1,273	n.s.
Interaction SxT500	0,288	2	0,144	2,763	n.s.
Interaction SxT501	0,028	2	0,014	0,27	n.s.
Error	2,866	55	0,052	0	

Table 32:Analysis of variance (ANOVA) for variable K the statistical differences of means aresignificant for p< .05 (*) F significant a 5% and p< .01(**) F sig at 1%

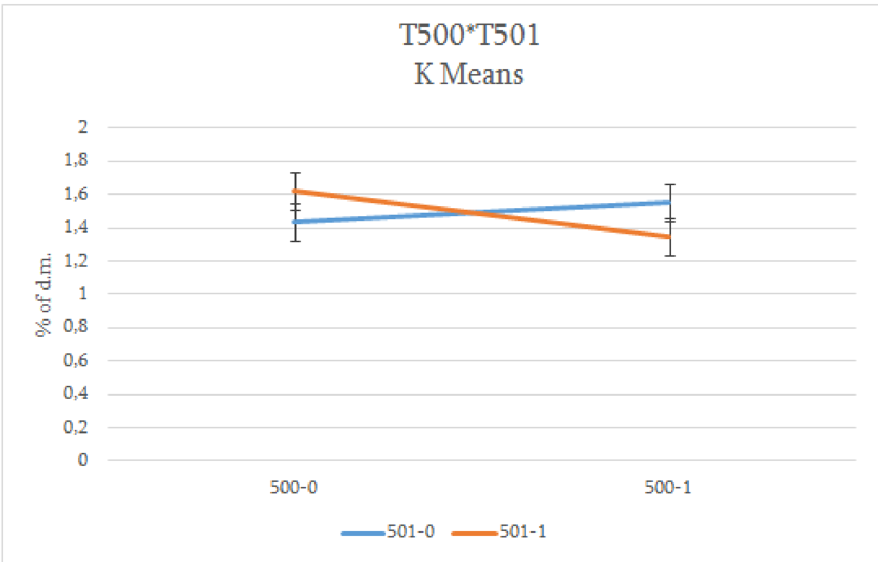

Figure 31:Graph showing the interaction between of 501 and 500 for the variable K.Significant for interaction 500 * 501 and level of K contents are lowest in absence oftreatment and variable when applied

K. Potassium show statistical significance for the interaction T500xT501. Striking were the low, almost in the region of shortfalls, Elf values (1.0 - 1.6% K of the dry matter for plant needs, the supply displayed with 1.56-1.92% was sufficient).

Dependent Variable: MgO					
Source	Sum of squares	df	Mean square	F	Sig.
Year (Y)	0,025	1	0,025	5,061	n.s.
Site (S)	0,322	2	0,161	19,931	*
Block (B)	0,001	2	0	0,618	n.s.
Interaction YxS	0,011	2	0,006	7,803	**
Treatment 500 (T500)	0,001	1	0,001	0,701	n.s.
Treatment 501 (T501)	0,003	1	0,003	1,548	n.s.
Interaction T500xT501	0,002	1	0,002	2,635	n.s.
Interaction YxT500	0,001	1	0,001	0,942	n.s.
Interaction YxT501	0,00008	1	0,00008	0,118	n.s.
Interaction SxT500	0,003	2	0,001	1,794	n.s.
Interaction SxT501	0,005	2	0,003	3,73	*
Error	0,039	55	0,001	0	

Table 33:Analysis of variance (ANOVA) for variable MgO the statistical differences of means are significant for p< .05 (*) F significant a 5% and p< .01(**) F sig at 1%

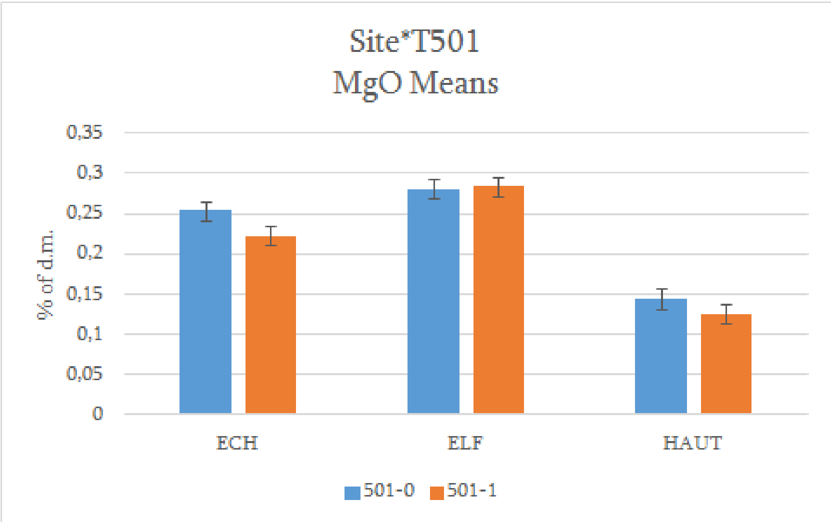

Figure 32:Bar graph showing the interaction between of 501 and Site for the variable MgO. Aneffect is shown only in Elf leaves. Ench and Haut decreased their contents after treatment.

Mg. Mainly regional and per regional per year magnesium contents show an influence after the treatment 501. In the table the treatment results are seen, the quantities in Elf remained the same after treatment, in Haut Ech the 501 treatment lowered them.

Dependent Variable: Ca					
Source	Sum of squares	df	Mean square	F	Sig.
Year (Y)	0,001	1	0,001	0,001	n.s.
Site (S)	0,926	2	0,463	0,936	n.s.
Block (B)	0,418	2	0,209	2,888	n.s.
Interaction YxS	0,746	2	0,373	5,152	**
Treatment 500 (T500)	0,086	1	0,086	1,128	n.s.
Treatment 501 (T501)	0,128	1	0,128	0,327	n.s.
Interaction T500xT501	0,007	1	0,007	0,097	n.s.
Interaction YxT500	0,079	1	0,079	1,084	n.s.
Interaction YxT501	0,267	1	0,267	3,692	n.s.
Interaction SxT500	0,14	2	0,07	0,966	n.s.
Interaction SxT501	0,393	2	0,196	2,71	n.s.
Error	3,984	55	0,072	0	

Table 34:Analysis of variance (ANOVA) for variable Ca the statistical differences of means aresignificant for p< .05 (*) F significant a 5% and p< .01(**) F sig at 1%

Ca. No statistical significance in calcium content, however according to reference is lower than a good care of plant.

Dependent Variable: Bo					
Source	Sum of squares	df	Mean square	F	Sig.
Year (Y)	4201,389	1	4201,389	391,609	n.s.
Site (S)	5034,194	2	2517,097	62,056	n.s.
Block (B)	73,444	2	36,722	0,577	n.s.
Interaction YxS	268,528	2	134,264	2,109	n.s.
Treatment 500 (T500)	1,389	1	1,389	0,02	n.s.
Treatment 501 (T501)	68,056	1	68,056	1,07	n.s.
Interaction T500xT501	8	1	8	0,126	n.s.
Interaction YxT500	0,222	1	0,222	0,003	n.s.
Interaction YxT501	3,556	1	3,556	0,056	n.s.
Interaction SxT500	16,361	2	8,181	0,129	n.s.
Interaction SxT501	50,861	2	25,431	0,399	n.s.
Error	3501,111	55	63,657	0	

Table 35:Analysis of variance (ANOVA) for variable Bo the statistical differences of means are significant for p< .05 (*) F significant a 5% and p< .01(**) F sig at 1%

Dependent Variable: Fe

Source	Sum of squares	df	Mean square	F	Sig.
Year (Y)	181704	1	181704	59,742	n.s.
Site (S)	6171,75	2	3085,875	0,378	n.s.
Block (B)	6339,25	2	3169,625	1,537	n.s.
Interaction YxS	2190,028	2	1095,014	0,531	n.s.
Treatment 500 (T500)	847,347	1	847,347	0,129	n.s.
Treatment 501 (T501)	2438,347	1	2438,347	0,372	n.s.
Interaction T500xT501	2977,347	1	2977,347	1,444	n.s.
Interaction YxT500	3321,125	1	3321,125	1,61	n.s.
Interaction YxT501	2750,347	1	2750,347	1,334	n.s.
Interaction SxT500	10650,86	2	5325,431	2,582	n.s.
Interaction SxT501	11746,19	2	5873,097	2,848	n.s.
Error	113437	55	2062,496	0	

Table 35:Analysis of variance (ANOVA) for variable Fe the statistical differences of means aresignificant for p< .05 (*) F significant a 5% and p< .01(**) F sig at 1%

Dependent Variable: Mn

Source	Sum of squares	df	Mean square	F	Sig.
Year (Y)	34584,5	1	34584,5	1,506	n.s.
Site (S)	5391,75	2	2695,875	0,064	n.s.
Block (B)	108	2	54	0,016	n.s.
Interaction YxS	56784,08	2	28392	8,37	**
Treatment 500 (T500)	4,5	1	4,5	0,001	n.s.
Treatment 501 (T501)	3280,5	1	3280,5	0,412	n.s.
Interaction T500xT501	16380,5	1	16380,5	4,829	*
Interaction YxT500	24,5	1	24,5	0,007	n.s.
Interaction YxT501	1334,722	1	1334,722	0,393	n.s.
Interaction SxT500	21064,08	2	10532	3,105	n.s.
Interaction SxT501	20023,08	2	10011,54	2,951	n.s.
Error	186575	55	3392,278	0	

Table 36:Analysis of variance (ANOVA) for variable Mn the statistical differences of means aresignificant for p< .05 (*) F significant a 5% and p< .01(**) F sig at 1%

Dependent Variable: Zn

Source	Sum of squares	df	Mean square	F	Sig.
Year (Y)	485,681	1	485,681	2,858	n.s.
Site (S)	87,028	2	43,514	0,16	n.s.
Block (B)	90,111	2	45,056	0,558	n.s.
Interaction YxS	600,028	2	300,014	3,716	*
Treatment 500 (T500)	276,125	1	276,125	69,175	n.s.
Treatment 501 (T501)	308,347	1	308,347	3,819	n.s.
Interaction T500xT501	165,014	1	165,014	2,044	n.s.
Interaction YxT500	30,681	1	30,681	0,38	n.s.
Interaction YxT501	0,681	1	0,681	0,008	n.s.
Interaction SxT500	108,083	2	54,042	0,669	n.s.
Interaction SxT501	159,361	2	79,681	0,987	n.s.
Error	4440,181	55	80,731	0	

Table 37:Analysis of variance (ANOVA) for variable Zn the statistical differences of means aresignificant for p< .05 (*) F significant a 5% and p< .01(**) F sig at 1%

Dependent Variable: Cu					
Source	Sum of squares	df	Mean square	F	Sig.
Year (Y)	625391	1	625391	6,57	n.s.
Site (S)	154603	2	77301,25	0,962	n.s.
Block (B)	19853,18	2	9926,592	0,651	n.s.
Interaction YxS	124427	2	62213,47	4,079	*
Treatment 500 (T500)	240,901	1	240,901	0,026	n.s.
Treatment 501 (T501)	1960,423	1	1960,423	0,027	n.s.
Interaction T500xT501	10265,06	1	10265,06	0,673	n.s.
Interaction YxT500	15952	1	15952	1,046	n.s.
Interaction YxT501	47529,58	1	47529,58	3,117	n.s.
Interaction SxT500	17453,12	2	8726,562	0,572	n.s.
Interaction SxT501	79766,85	2	39883,42	2,615	n.s.
Error	838798	55	15250,88	0	

Table 38:Analysis of variance (ANOVA) for variable Cu the statistical differences of means aresignificant for p< .05 (*) F significant a 5% and p< .01(**) F sig at 1%

B, Fe, Zn, Mn, Cu. In the sheet-boron contents a significant step of the process but not the type or location of the interaction process was found. This Haut ranges lack of supply and Auv in scarce supply. Elf, Ech and are in the range of 25 to 40 supply sufficient mg boron per kg dry matter.

For the micronutrients Fe, Mn, and Cu (data not shown) showed no differences in location and method a good supply, only with Zn is the rather short supply in all locations (18.6-27.7 ppm with reference values of 30-60 ppm).

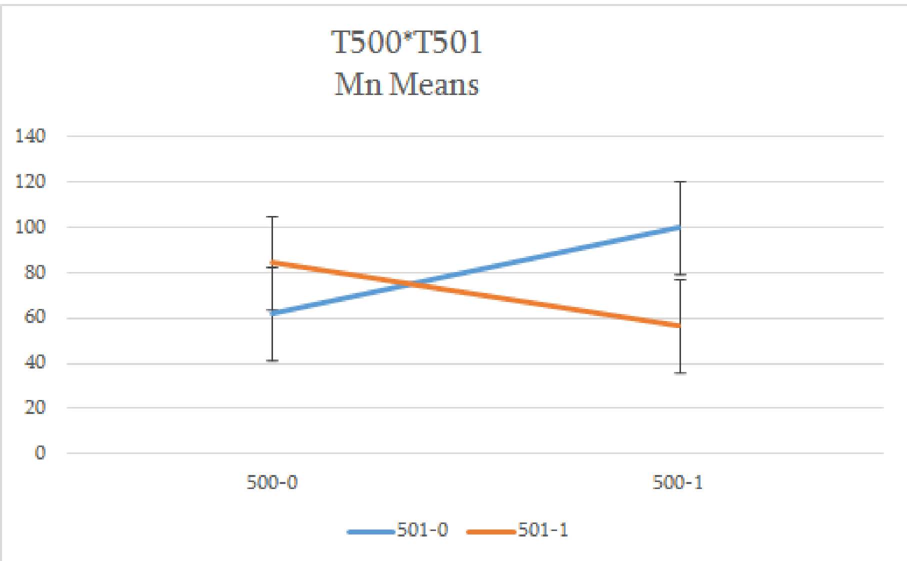

Figure 33:Graph showing the interaction between 500 and 501 for the variable Mn

3.2.1.1 SPAD Chlorophyll index

SPAD analysis carried in 2009 and 2010 show the following results:

Dependent Variable: SPAD

Source	Sum of squares	df	Mean square	F	Sig.
Year (Y)	13,621	1	13,621	0,179	n.s.
Site (S)	237,143	2	118,572	1,554	n.s.
Block (B)	6,413	2	3,206	0,757	n.s.
Interaction YxS	141,066	2	70,533	16,66	**
Treatment 500 (T500)	35,584	1	35,584	2,253	n.s.
Treatment 501 (T501)	36,67	1	36,67	9,204	n.s.
Interaction T500xT501	0,443	1	0,443	0,105	n.s.
Interaction YxT500	12,991	1	12,991	3,068	n.s.
Interaction YxT501	1,031	1	1,031	0,244	n.s.
Interaction SxT500	14,07	2	7,035	1,662	n.s.
Interaction SxT501	14,374	2	7,187	1,697	n.s.
Error	232,859	55	4,234	0	

Table 39: Analysis of variance (ANOVA) for variable SPAD the statistical differences of means are significant for p< .05 (*) F significant a 5% and p< .01(**) F sig at 1%

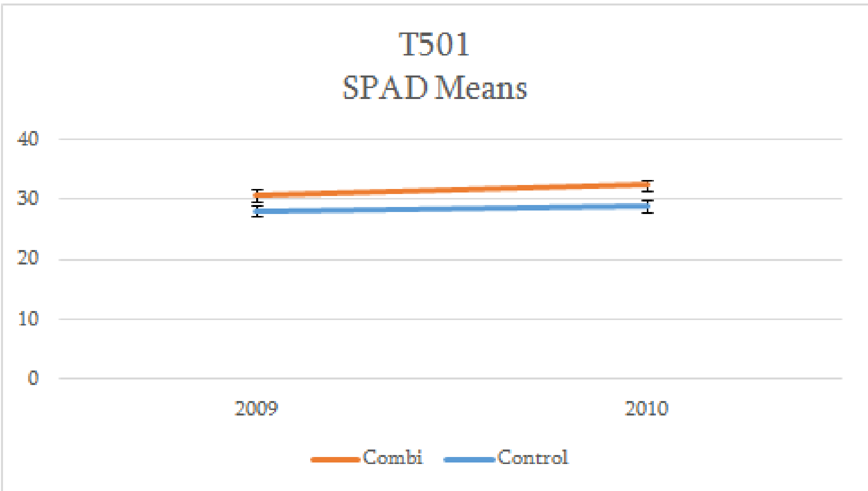

Figure 34: Graph showing the means of the treatments for the variable SPAD. Is not significantbut shows an interesting trend in Combi plots which show a higher SPAD value after 501treatment in Combi plots

SPAD. No statistical differences were observed except for the interaction Year*Site. Interesting, but not significant the graph above showing how 501 applied increased SPAD values in 2009 and 2010.

The interaction 501*Site nearly significant shows the highest SPAD levels with 501 on all sites. 501 without 500 has a very high SPAD; this may be due to an excess of stress in the high region of the plant.

3.2.3 Wood weight

The weight of winter pruning was measured.

Dependent Variable: Ww

Source	Sum of squares	df	Mean square	F	Sig.
Year (Y)	0	1	0	0,021	n.s.
Site (S)	0,093	2	0,047	1,367	n.s.
Block (B)	0,012	2	0,006	1,526	n.s.
Interaction YxS	0,044	2	0,022	5,872	**
Treatment 500 (T500)	0,004	1	0,004	0,267	n.s.
Treatment 501 (T501)	0,007	1	0,007	1,75	n.s.
Interaction T500xT501	0,007	1	0,007	1,856	n.s.
Interaction YxT500	0	1	0	0,03	n.s.
Interaction YxT501	0,000007	1	0,00007	0,018	n.s.
Interaction SxT500	0,039	2	0,019	5,145	**
Interaction SxT501	0,000008	2	0,000008	0,021	n.s.
Error	0,208	55	0,004	0	

Table 40:Analysis of variance (ANOVA) for variable Wood Weight thestatistical differences of means are significant for p< .05 (*) F significant a5% and p< .01(**) F sig at 1%

Figure 35:Graph showing the interaction between 500 and site for the variable woodweight. Shows an increase of woodweight only in Ech. Elf and Haut show a decrease in500 plots.

Figure 36:Graph showing the interaction between site and 501 for the variable woodweight. Not significant but the 501 stimulates woodweight. Results expressed in Kg dry matter.

Wood weight. Statistical analysis has evidence a highly significant interaction Year*Site and 500*Site as shown in the graph above, treatment 500 reduced the weight of winter pruning in Elf and Haut, in Ech there was an increase. Not significant, but it can help in the interpretation of data figure 24 where treatment 501 increased the wood weight in all three sites. Considered along with SPAD values the 501 wood weight values increase plant growth.

3.2.4 Single site statistical analysis

Results are influences by the different environmental condition, for this reason we decide to do a uni-variate analysis for single site for SPAD, Ww, N, P for the three sites (Exclude Auv without the 501 plot).

3.2.4.1 SPAD

SPAD. The single site analysis, significant in Haut for the use of 500, affects negatively the level of SPAD values. This may be due to a higher containment of plant metabolism. In Haut and Ech the interaction of 500*501 was significant and highly significant showing in Ech a middle value balance after use of both preparations. In Haut the no use of treatment in the Control plot shows a higher level.

Dependent Variable: SPAD site ECH

Source	Sum of squares	df	Mean square	F	Sig.
Year (Y)	0,501	1	0,501	0,044	n.s.
Block (B)	8,02	2	4,01	0,976	n.s.
Treatment 500 (T500)	0,156	1	0,156	0,01	n.s.
Treatment 501 (T501)	42,312	1	42,312	135,921	n.s.
Interaction T500xT501	41,256	1	41,256	10,045	**
Interaction YxT500	15,147	1	15,147	3,688	n.s.
Interaction YxT501	0,311	1	0,311	0,076	n.s.
Error	61,61	15	4,107	0	

Table 40:Analysis of variance (ANOVA) for variable SPAD in Ech the statistical differences ofmeans are significant for p< .05 (*) F significant a 5% and p< .01(**) F sig at 1%

Dependent Variable: SPAD site HAUT

Source	Sum of squares	df	Mean square	F	Sig.
Year (Y)	134,111	1	134,111	57,906	n.s.
Block (B)	0,407	2	0,204	0,088	n.s.
Treatment 500 (T500)	22,298	1	22,298	470,348	*
Treatment 501 (T501)	1,852	1	1,852	27,701	n.s.
Interaction T500xT501	20,289	1	20,289	8,762	*
Interaction YxT500	0,047	1	0,047	0,02	n.s.
Interaction YxT501	0,067	1	0,067	0,029	n.s.
Error	34,734	15	2,316	0	

Table 41:Analysis of variance (ANOVA) for variable SPAD in Haut the statistical differences ofmeans are significant for p< .05 (*) F significant a 5% and p< .01(**) F sig at 1%

Dependent Variable: SPAD site ELF					
Source	Sum of squares	df	Mean square	F	Sig.
Year (Y)	20,075	1	20,075	3,918	n.s.
Block (B)	41,231	2	20,616	13,244	**
Treatment 500 (T500)	27,2	1	27,2	5,978	n.s.
Treatment 501 (T501)	6,88	1	6,88	3,23	n.s.
Interaction T500xT501	0,586	1	0,586	0,376	n.s.
Interaction YxT500	4,55	1	4,55	2,923	n.s.
Interaction YxT501	2,13	1	2,13	1,368	n.s.
Error	23,349	15	1,557		

Table 41:Analysis of variance (ANOVA) for variable SPAD in Elf the statistical differences ofmeans are significant for p< .05 (*) F significant a 5% and p< .01(**) F sig at 1%

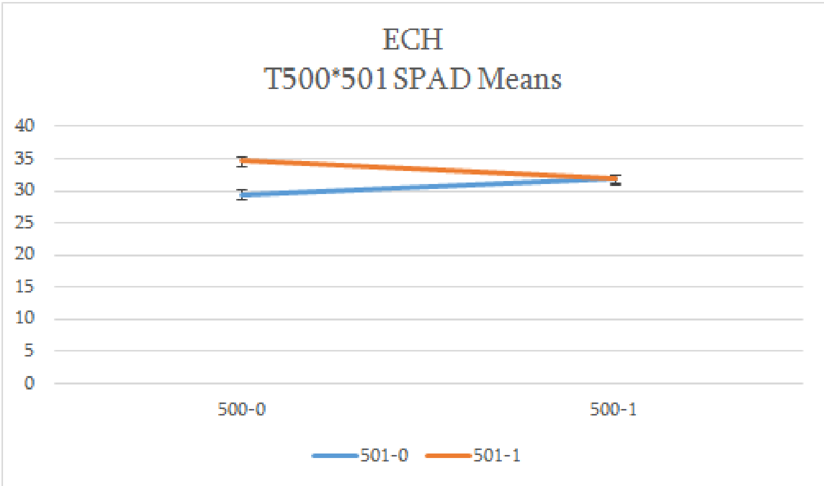

Figure 37:Bar graph showing the interaction between 500 and 501 for the variable SPAD in Echandens

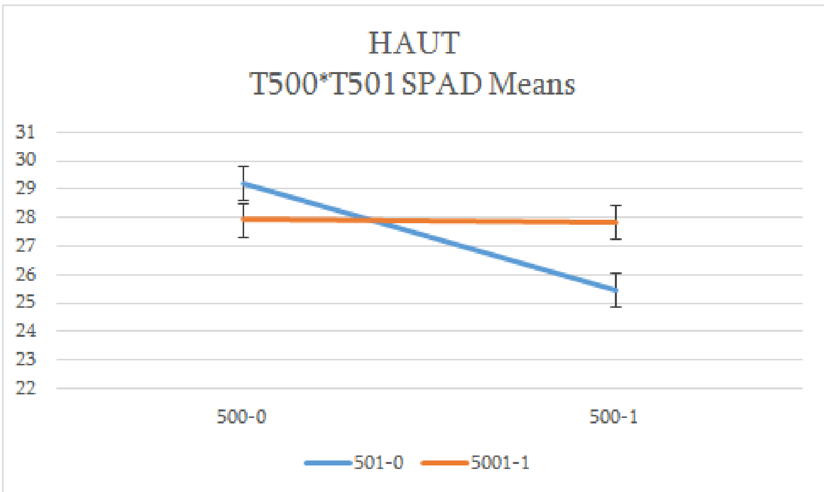

Figure 38: Graph showing the interaction between 500 and 501 for the variable SPAD in Hauterive

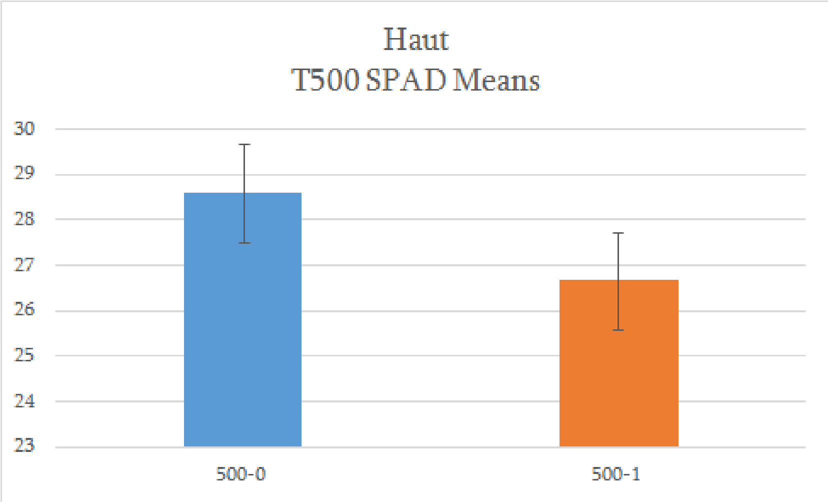

Figure 39:Bar graph showing the means of the treatments for the variable SPAD in Hauterive

3.2.4.2 Wood Weight and length

Dependent Variable: Ww Site ECH

Source	Sum of squares	df	Mean square	F	Sig.
Year (Y)	0,011	1	0,011	0,211806	n.s.
Block (B)	0,019	2	0,009	4,292	*
Treatment 500 (T500)	0,015	1	0,015	7,5	n.s.
Treatment 501 (T501)	0,003	1	0,003	3,449	n.s.
Interaction T500xT501	0,066	1	0,066	29,955	**
Interaction YxT500	0	1	0	0	n.s.
Interaction YxT501	0,001	1	0,001	0,37	n.s.
Error	0,033	15	0,002	7,942	

Table 42:Analysis of variance (ANOVA) for variable Wood weight in Ech the statistical differences of means are significant for p< .05 (*) F significant a 5% and p< .01(**) F sig at 1%

Dependent Variable: Ww Site HAUT

Source	Sum of squares	df	Mean square	F	Sig.
Year (Y)	0,026	1	0,026	7,942	n.s.
Block (B)	0,001	2	0	0,803	n.s.
Treatment 500 (T500)	0,018	1	0,018	6,76	n.s.
Treatment 501 (T501)	0,003	1	0,003	2,163	n.s.
Interaction T500xT501	0,000002	1	0,000002	0,008	n.s.
Interaction YxT500	0,003	1	0,003	4,875	*
Interaction YxT501	0,001	1	0,001	2,254	n.s.
Error	0,008	15	0,001	0	

Table 43:Analysis of variance (ANOVA) for variable Wood weight in Haut the statistical differences of means are significant for p< .05 (*) F significant a 5% and p< .01(**) F sig at 1%

Dependent Variable: Ww Site ELF					
Source	Sum of squares	df	Mean square	F	Sig.
Year (Y)	0,007	1	0,007	0,884	n.s.
Block (B)	0,034	2	0,017	6,711	**
Treatment 500 (T500)	0,01	1	0,01	2,163	n.s.
Treatment 501 (T501)	0,001	1	0,001	0,224	n.s.
Interaction T500xT501	0,012	1	0,012	4,831	*
Interaction YxT500	0,005	1	0,005	1,915	n.s.
Interaction YxT501	0,006	1	0,006	2,392	n.s.
Error	0,038	15	0,003		

Table 44:Analysis of variance (ANOVA) for variable Wood weight in Elf the statistical differences of means are significant for p< .05 (*) F significant a 5% and p< .01(**) F sig at 1%

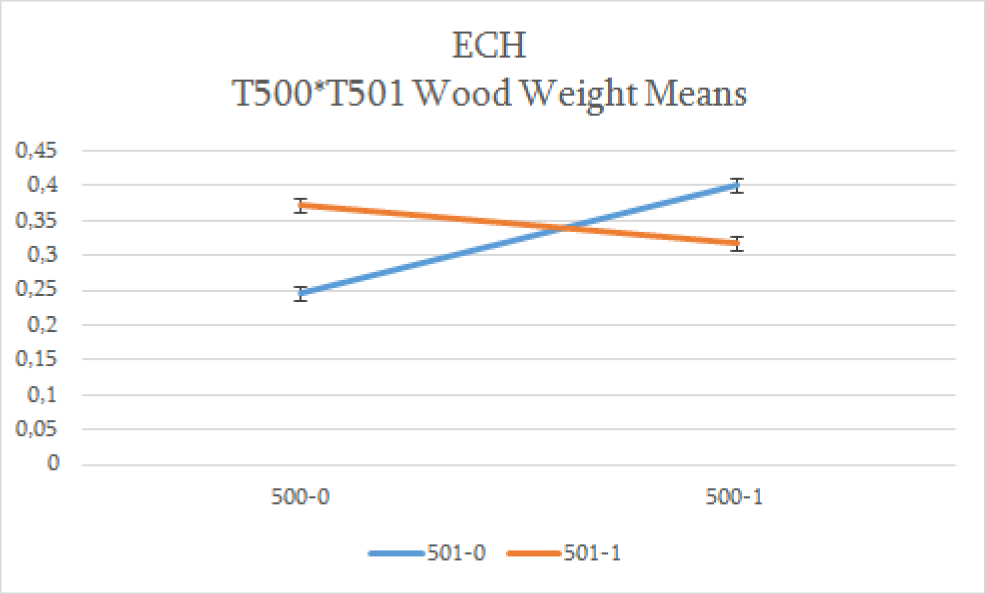

Figure 40:Graph showing the interaction between 500 and 501 for the variable wood weight in Echandens

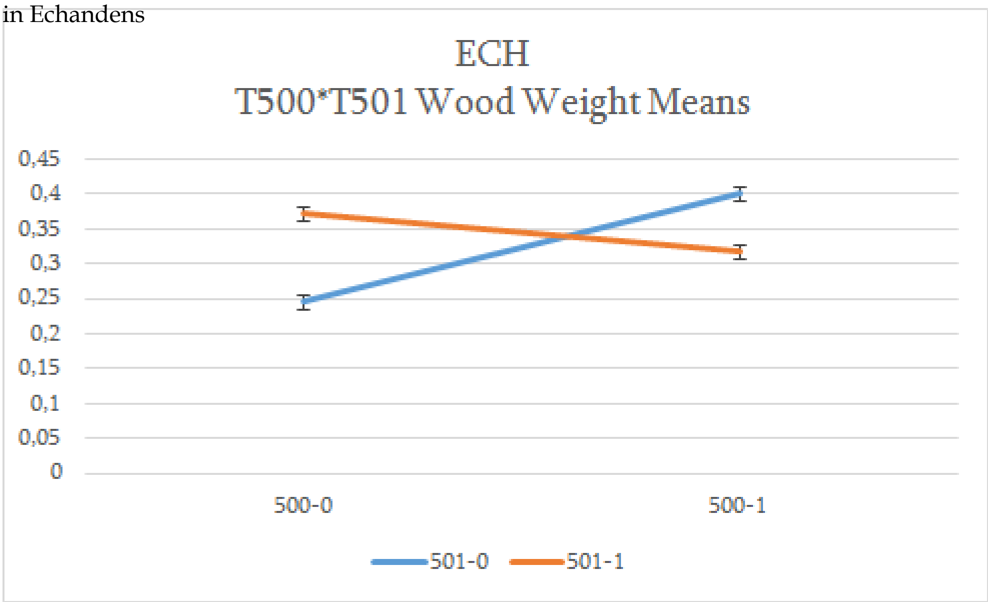

Figure 41:Graph showing the interaction between 500 and 501 for the variable wood weight in Elfingen

Wood Weight. In Elf and Ech the interaction 500*501 showed significant and highly significant results, as shown in the graphs the same trend is observed. The use of at least one of the two preparations increased wood weight (0.4 kg/ vine), while the non use (Control plot) reduced wood weight. With the use of 500 without 501 the wood weight is higher in Ech, while Elf showed a higher value for 500 plus 501.

Dependent Variable: Length

Source	Sum of squares	df	Mean square	F	Sig.
T500	58,906	1	58,906	0,905	n.s.
T501	29,976	1	29,976	0,461	n.s.
Site	1116,397	3	372,132	5,718	*
T500 * T501	300,156	1	300,156	4,612	n.s.
Error	585,751	9	65,083	0	

Table 45:Analysis of variance (ANOVA) for variable Length statistical differences of means are significant for $p< .05$ (*) F significant a 5% and $p< .01$(**) F sig at 1%

Length. No statistical results were obtained for the length model, except for the site. As with the wood weight, the length average show a better growth in the process 501 but not a statistical influence (data not shown). The 500 treatment tends to reduce length, but this is odd as it should stimulate growth, the interaction borders statistical significance for $p < 0.054$. When 500 is absent length is not promoted, does 501 in absence of 500 promote length?

The wood weight is the weight of the shoot, the power, hence 501 lengthens a line of shoots, but it doesn't make them fray at a vegetative level. 501 stresses them as they compete shooting upwards.

3.2.4.3 N and P in the leaves

Dependent Variable: N Site ECH

Source	Sum of squares	df	Mean square	F	Sig.
Year (Y)	0,006	1	0,006	0,508	n.s.
Block (B)	0,006	2	0,003	0,325	n.s.
Treatment 500 (T500)	0,000006	1	0,000006	0	n.s.
Treatment 501 (T501)	0,044	1	0,044	5,095	n.s.
Interaction T500xT501	0,116	1	0,116	13,017	**
Interaction YxT500	0,012	1	0,012	1,324	n.s.
Interaction YxT501	0,009	1	0,009	0,965	n.s.
Error	0,134	15	0,009	0	

Table 46:Analysis of variance (ANOVA) for variable N in Ech the statistical differences ofmeans are significant for p< .05 (*) F significant a 5% and p< .01(**) F sig at 1%

Dependent Variable: N Site HAUT

Source	Sum of squares	df	Mean square	F	Sig.
Year (Y)	0,028	1	0,028	2,54	n.s.
Block (B)	0,001	2	0,001	0,077	n.s.
Treatment 500 (T500)	0	1	0	1,817	n.s.
Treatment 501 (T501)	0,003	1	0,003	0,186	n.s.
Interaction T500xT501	0,041	1	0,041	5,516	*
Interaction YxT500	0	1	0	0,026	n.s.
Interaction YxT501	0,018	1	0,018	2,424	n.s.
Error	0,113	15	0,008	0	

Table 47:Analysis of variance (ANOVA) for variable N in Haut the statistical differences ofmeans are significant for p< .05 (*) F significant a 5% and p< .01(**) F sig at 1%

Dependent Variable: N Site ELF

Source	Sum of squares	df	Mean square	F	Sig.
Year (Y)	0	1	0	0	n.s.
Block (B)	0,009	2	0,004	0,455	n.s.
Treatment 500 (T500)	0,092	1	0,092	19,703	n.s.
Treatment 501 (T501)	0,004	1	0,004	2,99	n.s.
Interaction T500xT501	0,004	1	0,004	0,445	n.s.
Interaction YxT500	0,005	1	0,005	0,497	n.s.
Interaction YxT501	0,001	1	0,001	0,145	n.s.
Error	0,141	15	0,009	0,471	

Table 48:Analysis of variance (ANOVA) for variable N in Elf the statistical differences ofmeans are significant for p< .05 (*) F significant a 5% and p< .01(**) F sig at 1%

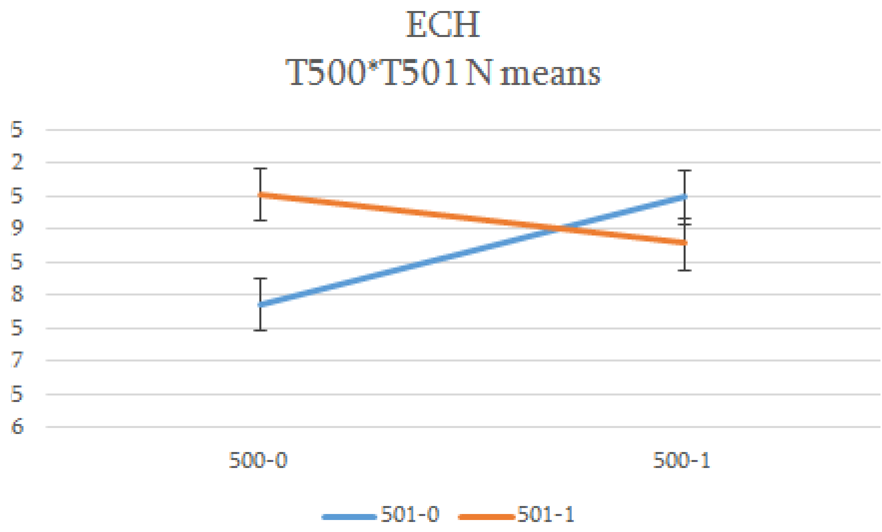

Figure 42: Graph showing the interaction between 500 and 501 for the variable N in Ech

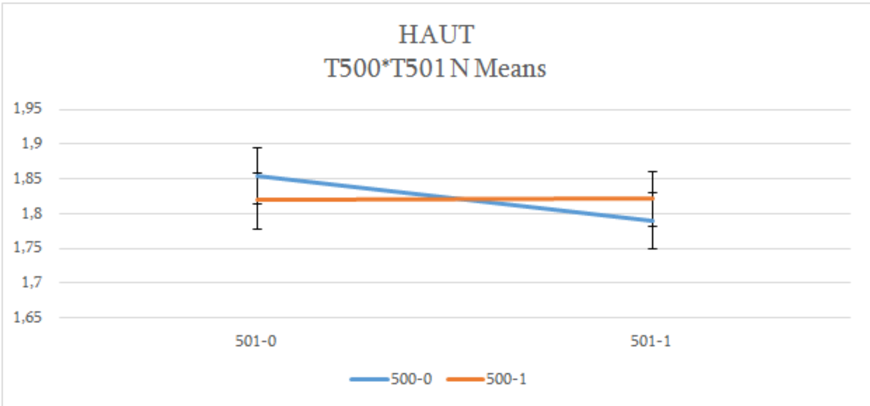

Figure 43: Graph showing the interaction between 500 and 501 for the variable N in Haut

N. Also here significant results were obtained in Ech and Haut with evidence of statistical significance and high statistical significance for the 500*501 interaction, as seen in the tables the trend is the same followed by SPAD.

Dependent Variable: P site ECH

Source	Sum of squares	df	Mean square	F	Sig.
Year (Y)	0,002	1	0,002	0,471	n.s.
Block (B)	0,001	2	0,001	0,653	n.s.
Treatment 500 (T500)	0,00004	1	0,0004	0,009	n.s.
Treatment 501 (T501)	0,009	1	0,009	20,694	n.s.
Interaction T500xT501	0,005	1	0,005	4,652	*
Interaction YxT500	0,005	1	0,005	4,542	n.s.
Interaction YxT501	0	1	0	0,434	n.s.

Table 49:Analysis of variance (ANOVA) for variable P in Ech the statistical differences ofmeans are significant for p< .05 (*) F significant a 5% and p< .01(**) F sig at 1%

Dependent Variable: P Site HAUT

Source	Sum of squares	df	Mean square	F	Sig.
Year (Y)	0,08	1	0,08	118,409	n.s.
Block (B)	0,001	2	0,001	3,519	n.s.
Treatment 500 (T500)	0,003	1	0,003	5,009	n.s.
Treatment 501 (T501)	0,001	1	0,001	3,449	n.s.
Interaction T500xT501	0,011	1	0,011	57,752	**
Interaction YxT500	0,001	1	0,001	3,417	n.s.
Interaction YxT501	0	1	0	1,055	n.s.
Error	0,003	15	0	0	

Table 50:Analysis of variance (ANOVA) for variable P in Haut the statistical differences ofmeans are significant for p< .05 (*) F significant a 5% and p< .01(**) F sig at 1%

Dependent Variable: P Site ELF

Source	Sum of squares	df	Mean square	F	Sig.
Year (Y)	0,004	1	0,004	4	n.s.
Block (B)	0,002	2	0,001	1,857	n.s.
Treatment 500 (T500)	0,009	1	0,009	9	n.s.
Treatment 501 (T501)	0,004	1	0,004	6162,25	**
Interaction T500xT501	0,001	1	0,001	1,027	n.s.
Interaction YxT500	0	1	0	0	n.s.
Interaction YxT501	0,000001	1	0,000001	0,001	n.s.
Error	0,008	15	0,001	0	

Table 51:Analysis of variance (ANOVA) for variable P in Elf the statistical differences ofmeans are significant for p< .05 (*) F significant a 5% and p< .01(**) F sig at 1%

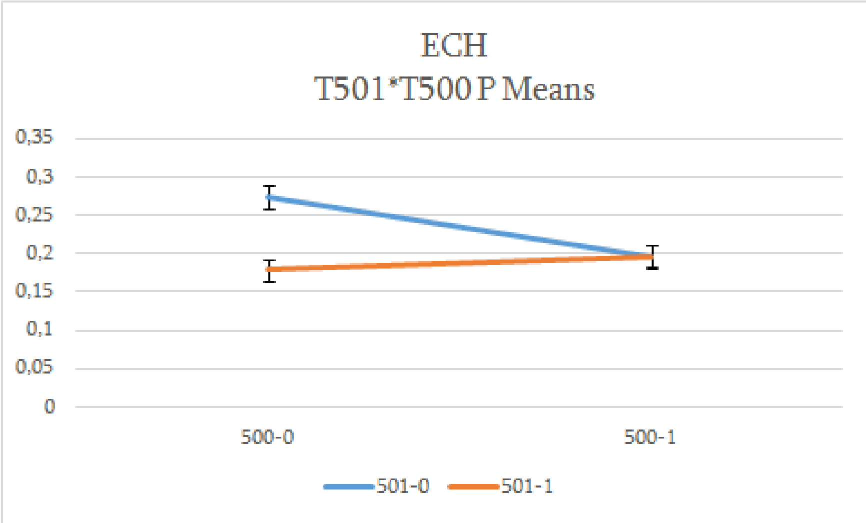

Figure 44:Analysis of variance (ANOVA) for variable P in Ech the statistical differences ofmeans are significant for p< .05 (*) F significant a 5% and p< .01(**) F sig at 1%

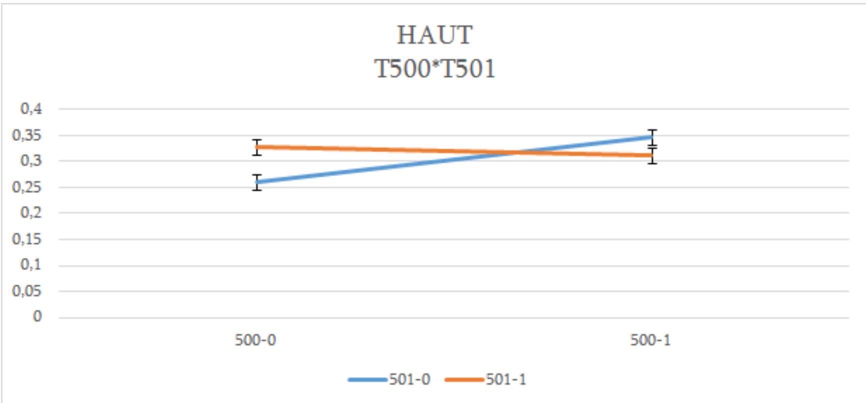

Figure 45:Analysis of variance (ANOVA) for variable P in Haut the statistical differences ofmeans are significant for p< .05 (*) F significant a 5% and p< .01(**) F sig at 1%

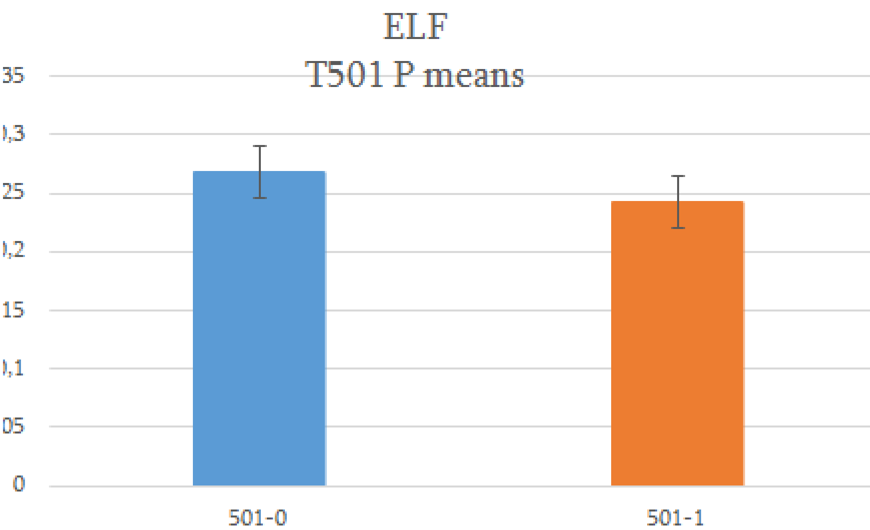

Figure 46:Analysis of variance (ANOVA) for variable P in Elf the statistical differences ofmeans are significant for p< .05 (*) F significant a 5% and p< .01(**) F sig at 1%

P. Highly significant results were obtained in Elf for treatment 501 that reduced the levels of potassium in leaves. In Haut and Ech significant and highly significant was the interaction 500*501 as shown in the figure above the use of no treatment in Ech shows a higher level of potassium, with 500 the value remained unchanged regardless of the use or non use of 501.

3.2.5 Disease incidence and severity

In 2008 no occurrence of Oidium was observed for all sites and plots. On the other hand a high pressure of Mildew disease was observed on 60-80% on the leaves. No statistical differences were observed between the plots and sites. Concerning the grapes, at the harvest, 50 % of the crop was lost for Auvernier as the farm management had decided not to apply any treatment (for other sites data not shown). No statistical differences were shown between the treatment plots. In 2009-2010 in the four sites and plots no disease observed (whether Oidium or Mildew).

3.2.5.1 Phytoalexin in the plant

The extremely bad season in 2008, created a stressful situation for the plants, the result was an increase of production of phytoalexins.

Dependent Variable: Sum Phytoalexins

Source	Sum of squares	df	Mean square	F	Sig.
T500	588,467	1	588,467	4,931	n.s.
T501	2037,77	1	2037,77	17,076	**
Site	2514,539	3	838,18	7,024	*
T500 * T501	207,6	1	207,6	1,74	n.s.
Error	1074,016	9	119,335	0	

Table 52:Analysis of variance (ANOVA) for variable Sum Phytoalexins in the statistical differences of means are significant for p< .05 (*) F significant a 5% and p< .01(**) F sig at 1%

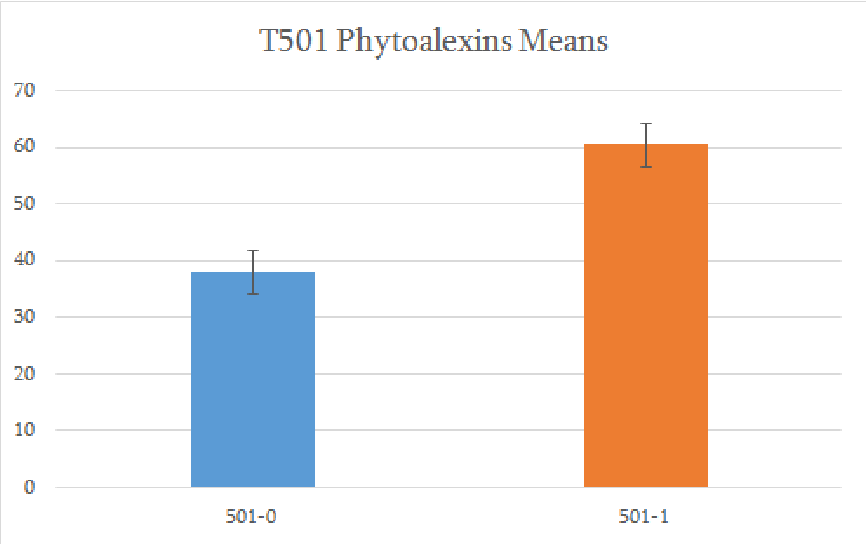

Figure 47: Bar graph showing the means of the treatments 501 for the variable Phytoalexins

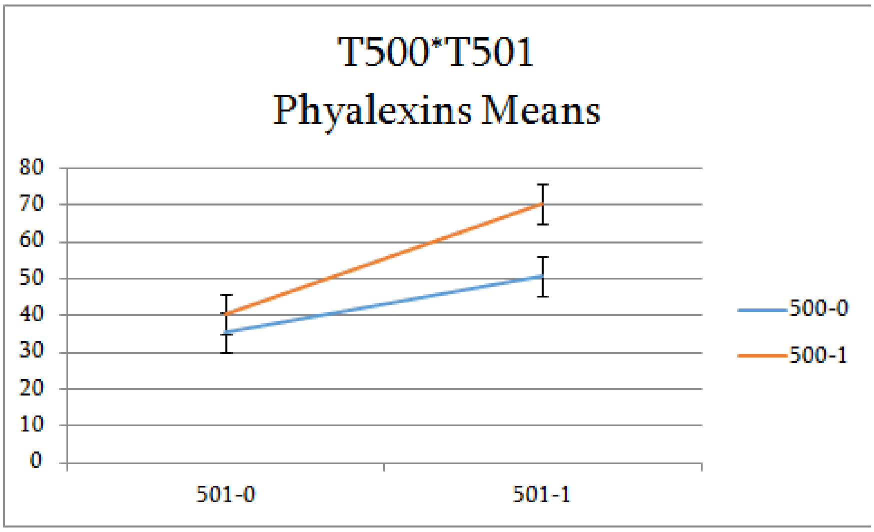

Figure 48:Graph showing the interaction between 500 and 501 for the variable Phytoalexins but with no significant statistical differences. This can help understanding the treatment f 501.

Phytoalexin. With treatment 501 the levels of phytoalexin are statistically significant. The graph shows how the application of 501 increases considerably the phytoalexin content increasing the immune system of the plant. For a better understanding of the phenomenon the second graph, though not significant, shows that the joint application of 500 and 501 has as a result a higher value. The non use of 501 has shown in both cases a lower level of phytoalexin.

3.3 Grapes

3.3.1 Yield

Dependent variable: Yield

Source	Sum of squares	df	Mean square	F	Sig.
Year	1,121	1	1,121	282,352	*
Treat	0,078	1	0,078	19,589	n.s
Treat * Year	0,004	1	0,004	0,022	n.s
Error	2,145	12	0,179		

Table 53:Analysis of variance (ANOVA) for variable Yield the statistical differences of means are significant for p< .05 (*) F significant a 5% and p< .01(**) F sig at 1%

In 2009 and 2010 were measured the kg m 2-1 from the rows treated Combi and Control, but no significant statistical differences were found. The Control plot produces less compared to the other years but it is not significant.

3.3.2 Quality of musts

In the years 2008- 2010 must analysis was carried out for plots Combi and Control.

Dependent variable: acidityMust

Source	Sum of squares	df	Mean square	F	Sig.
Year	32,572	2	16,286	47,622	**
Site	3,58	3	1,193	9,989	*
Treatment	0,217	1	0,217	1,816	n.s.
Treatment * Site	0,359	3	0,12	0,36	n.s.
Error	4,658	14	0,333		

Table 54: Analysis of variance (ANOVA) for variable acidity must the statistical differences of means are significant for p< .05 (*) F significant a 5% and p< .01(**) F sig at 1%

Source	Sum of squares	df	Mean square	F	Sig.
Year	0,258	2	0,129	12,56	**
Site	0,031	3	0,01	16,851	*
Treatment	0,00003	1	0,00003	0,064	n.s.
Treatment * Site	0,002	3	0,001	0,059	n.s.
Error	0,147	14	0,01		

Table 55: Analysis of variance (ANOVA) for variable pH must the statistical differences of means are significant for p< .05 (*) F significant a 5% and p< .01(**) F sig at 1%

In 2008 - 2010 must analysis shows statistical significance only for year and site. The qualitative parameters of freshly, squeezed musts were very similar between the different methods (data not shown). The sugar contents in 2009 Ech (86 Oe) and Auv (86.5 Oe), despite the relatively high yields were significantly higher than the cantonal average values of 75 degrees Oechsle. Likewise, in this Elf with Riesling x Sylvaner was the case (78.5 Oe).

At all sites, the acid contents were high, the musts would be protected against oxidation processes during fermentation.

Significant procedural differences were not evident in any of the locations.

Kinetics and execution of fermentation

The good state of health of the grapes in 2008-2010 allowed a low SO2 dose of 25 ml / l. Depending on the experimental site, the sugar contents were different. Haut and Elf were slightly enriched with 1 kg, respectively, and 0.5 kg/100 L. Auv and Ech showed the highest maturity with values of 86 ° and 87 ° Oechsle and were not chaptalised.

Alcoholic fermentation 2008-2010

The alcoholic fermentation was smooth for all plots and sites, the sugar was completely converted. All must parameters in the standard quality of the grapes.

The malo-lactic fermentation was researched and achieved for all location and variants at the end of the winter. The tartaric precipitation was realized with the natural cold of winter (-8C during 3 weeks). Concerning the protein stabilization no fining product (bentonite, etc) was added and filtration.

The bottling was begun in spring of each year.

In the course of fermentation no differences between the Control and Combination method could be observed.

3.4 Wine analytic analyses

Dependent variable: Alcool

Source	Sum of squares	df	Mean square	F	Sig.
Year	0,869	2	0,434	1,899	n.s.
Site	1,335	3	0,445	21,659	*
Treatment	0,03	1	0,03	1,46	n.s.
Treatment * Site	0,062	3	0,021	0,09	n.s.
Error	3,217	14	0,23		

Table 56: Analysis of variance (ANOVA) for variable alcohol the statistical differences of means are significant for p< .05 (*) F significant a 5% and p< .01(**) F sig at 1%

Dependent variable: Density

Source	Sum of squares	df	Mean square	F	Sig.
Year	6,40E-006	2	3,20E-006	6,195	*
Site	4,28E-006	3	1,43E-006	2,392	n.s.
Treatment	3,63E-007	1	3,63E-007	0,608	n.s.
Treatment * Site	1,79E-006	3	5,97E-007	1,193	n.s.
Error	7,00E-006	14	5,00E-007		

Table 57: Analysis of variance (ANOVA) for variable density the statistical differences of means are significant for p< .05 (*) F significant a 5% and p< .01(**) F sig at 1%

Dependent variable: Fructose

Source	Sum of squares	df	Mean square	F	Sig.
Year	6,194	2	3,097	2,187	n.s.
Site	3,725	3	1,242	0,951	n.s.
Treatment	0,217	1	0,217	0,166	n.s.
Treatment * Site	3,918	3	1,306	0,919	n.s.
Error	19,905	14	1,422		

Table 58: Analysis of variance (ANOVA) for variable fructose the statistical differences of means are significant for p< .05 (*) F significant a 5% and p< .01(**) F sig at 1%

Dependent variable: Totalac

Source	Sum of squares	df	Mean square	F	Sig.
Year	1,002	2	0,501	1,72	n.s.
Site	3,25	3	1,083	53,875	**
Treatment	0,113	1	0,113	5,605	n.s.
Treatment * Site	0,061	3	0,02	0,072	n.s.
Error	3,976	14	0,284		

Table 59: Analysis of variance (ANOVA) for variable total acidity the statistical differences of means are significant for p< .05 (*) F significant a 5% and p< .01(**) F sig at 1%

Dependent variable: Aceticac

Source	Sum of squares	df	Mean square	F	Sig.
Year	0,002	2	0,001	0,174	n.s.
Site	0,012	3	0,004	0,893	n.s.
Treatment	0	1	0	0,051	n.s.
Treatment * Site	0,013	3	0,004	0,792	n.s.
Error	0,079	14	0,006		

Table 60: Analysis of variance (ANOVA) for variable acetic acid the statistical differences of means are significant for p< .05 (*) F significant a 5% and p< .01(**) F sig at 1%

Dependent variable: pH

Source	Sum of squares	df	Mean square	F	Sig.
Year	0,112	2	0,056	4,363	*
Site	0,114	3	0,038	3,242	n.s.
Treatment	0,006	1	0,006	0,492	n.s.
Treatment * Site	0,035	3	0,012	0,94	n.s.
Error	0,174	14	0,012		

Table 61: Analysis of variance (ANOVA) for variable pH the statistical differences of means are significant for p< .05 (*) F significant a 5% and p< .01(**) F sig at 1%

Dependent variable: Glycerol

Source	Sum of squares	df	Mean square	F	Sig.
Year	4,592	2	2,296	4,227	*
Site	4,565	3	1,522	2,078	n.s.
Treatment	0,675	1	0,675	0,922	n.s.
Treatment * Site	2,196	3	0,732	1,401	n.s.
Error	7,318	14	0,523		

Table 62: Analysis of variance (ANOVA) for variable glycerol the statistical differences of means are significant for p< .05 (*) F significant a 5% and p< .01(**) F sig at 1%

Dependent variable: lacticAc

Source	Sum of squares	df	Mean square	F	Sig.
Year	0,498	2	0,249	1,169	n.s.
Site	0,022	3	0,007	0,061	n.s.
Treatment	1,152	1	1,152	9,389	n.s.
Treatment * Site	0,368	3	0,123	0,563	n.s.
Error	3,056	14	0,218		

Table 63: Analysis of variance (ANOVA) for variable lactic acid the statistical differences of means are significant for p< .05 (*) F significant a 5% and p< .01(**) F sig at 1%

Dependent variable: volatileAc

Source	Sum of squares	df	Mean square	F	Sig.
Year	0,009	2	0,004	3,217	n.s.
Site	0,014	3	0,005	3,017	n.s.
Treatment	0,01	1	0,01	6,29	n.s.
Treatment * Site	0,005	3	0,002	1,18	n.s.
Error	0,019	14	0,001		

Table 64: Analysis of variance (ANOVA) for variable volatile acid the statistical differences of means are significant for p< .05 (*) F significant a 5% and p< .01(**) F sig at 1%

Dependent variable: malic Ac.

Source	Sum of squares	df	Mean square	F	Sig.
Year	1,714	2	0,857	1,537	n.s.
Site	1,665	3	0,555	2,546	n.s.
Treatment	1,459	1	1,459	6,69	n.s.
Treatment * Site	0,655	3	0,218	0,386	n.s.
Error	7,912	14	0,565		

Table 65: Analysis of variance (ANOVA) for variable malic acid the statistical differences of means are significant for p< .05 (*) F significant a 5% and p< .01(**) F sig at 1%
Table 66: Analysis of variance (ANOVA) for variable tartaric acid the statistical differences of means are significant for p< .05 (*) F significant a 5% and p< .01(**) F sig at 1%

Dependent variable: tartaricAc.

Source	Sum of squares	df	Mean square	F	Sig.
Year	0,726	2	0,363	8,677	**
Site	0,183	3	0,061	2,078	n.s.
Treatment	0,096	1	0,096	3,271	n.s.
Treatment * Site	0,088	3	0,029	0,705	n.s.
Error	0,585	14	0,042		

Table 66: Analysis of variance (ANOVA) for variable tartaric acid the statistical differences of means are significant for p< .05 (*) F significant a 5% and p< .01(**) F sig at 1%

In wine analyses for pH, acidity and alcohol no statistical differences were found from 2009-2011. A nearly statistical difference was noticed in the quantity of malic acid and lactic acid for the treatment plots. Malic acid seems to be lower in Combi plots while the lactic acid is higher. As described earlier, malo-lactic fermentation was desired, but the malic acid content was different.

As malic acid is a ripeness indicator a lower quantity in the wine and consequently in the grapes could indicate a better ripening process.

Concerning this aspect, a recent study by A. Duval-Chaboussou (not published yet) detected a variance in malic acid content the grape at harvest. In any case this fact needs further investigation and oenological competence.

Wine analytic analyses

Analyses	Alcool [%vol.]	Density / degree Oechsle	Fructose [g/l]	Glucose [g/l]	Total acidity [g/l]	Acetic acid [g/l]	pH	Glycerol [g/l]	sucrose [g/l]	lactic acid [g/l]	volatile acidity [g/l]	malic acid [g/l]	tartaric acid [g/l]
Auv2008 Control	11.6	0.993	0	0	6.99	0.2	3.33	6.9	0	0.2	0.4	3.3	2.8
Auv2008 Combi	11.64	0.993	0	0	6.11	0.3	3.38	7.5	0	2.1	0.5	0.7	2.3
Auv2009 Control	11.63	0.993	3	0	5.37	0.2	3.6	8.1	0	1.9	0.5	0.6	1.8
Auv2009 Combi	11.93	0.993	2	0	5.6	0.2	3.58	8.4	0	2.3	0.5	0.2	1.8
Auv2010 Control	11.67	0.992	1	0	5.24	0.2	3.58	7.8	0	1.2	0.4	1.6	1.9
Auv2010 Combi	11.69	0.992	1	0	4.8	0.1	3.48	6.4	0	2.1	0.5	0	1.7
Haut 2008 Control	10.95	0.992	1	0	4.78	0.1	3.55	6.7	0	2	0.5	0.3	1.9
Haut 2008 Combi	10.78	0.993	0	0	5.12	0.2	3.5	6.9	0	2.1	0.5	0.7	2
Haut 2009 Control	10.63	0.994	2	0	5.13	0.1	3.56	8.5	0	2.1	0.5	0.5	2
Haut 2009 Combi	10.78	0.993	1	0	4.81	0.1	3.6	8	0	2.1	0.5	0	1.7
Haut 2010 Control	11.33	0.991	0	0	4.88	0.1	3.3	5.6	0	1.9	0.5	0	2.1
Haut 2010 Combi	11.25	0.992	1	0	5.27	0.2	3.24	6.4	0	2	0.4	0	2.2
Elf 2008 Control	11.5	0.992	2	0	5.05	0.1	3.18	5.9	0	1.3	0.4	0.4	2.3
Elf 2008 Combi	11.65	0.992	4	0	5.13	0.2	3.15	6	0	1.6	0.4	0.1	2.3
Elf 2009 Control	11.06	0.993	2	1	5.79	0.2	3.36	7.4	0	1	0.5	1.6	2.3
Elf 2009 Combi	11.16	0.993	2	0	5.28	0.2	3.42	7.7	0	2.2	0.5	0.2	1.8
Elf 2010 Control	11.77	0.992	1	0	5.26	0.1	3.37	7.6	0	2.1	0.5	0.1	1.8
Elf 2010 Combi	11.89	0.992	1	2	5.27	0.2	3.39	7.4	0	1.7	0.5	0.4	1.9
Ech 2008 Control	11.12	0.993	0	2	5.19	0.2	3.46	5.8	0	1.5	0.4	1.2	2.2
Ech 2008 Combi	11.27	0.993	0	2	4.97	0.2	3.47	6.3	0	1.7	0.4	0.4	2.2

Figure 49: Wine analytical analyses for wines from 2008-2010.

3.4.1 Wine sensorial

3.4.1.1 Triangle test

Only in the 2009 wines and in particular in the Chasselas Ech the examiners were able to sample wines significantly different from each other.

Comparison Control Vs Combi	Total replies	Correct answers	Significant (p=0.05)
1 -Chasselas Haut Bio 08/ Biodyn 08	39	15	n.s.
2 -Chasselas Auv Bio 08/Biodyn 08	39	10	n.s.
3 - Chasselas Ech Bio 08/Biodyn 08	39	17	n.s.
4 -Riesling/Sylvaner Elf Bio 08/Biodyn 08	39	15	n.s.

Table 67: Triangle test with the microvinification BoBdyn and white wines of 2008 (March 8, 2010)

Number of examiners = 39

Comparison Control Vs Combi	Total replies	Correct answers	Significant (p=0.05)
1 -Chasselas Haut Bio 09/ Biodyn 09	40	15	n.s.
2 -Chasselas Auv Bio 09/Biodyn 09	40	16	n.s.
3 -Chasselas Ech Bio 09/Biodyn 09	40	33	***
4 -Riesling/Sylvaner Elf Bio09/Biodyn 09	40	17	n.s.

Table 68: Triangle test with the microvinification BoBdyn and white wines of 2009 (March 11, 2010); examiners number = 40

Comparison Control Vs Combi	Total replies	Correct answers	Significant (p=0.05)
1 -Haut 10 Control/Haut 10 Combi	31	13	n.s.
2 -Auv 10 Control/Auv 10 Combi	31	11	n.s.
3 -Echan 10 Control/Echan 10 Combi	31	8	n.s.
4 -RS Elfi 10 Control/RS Elfi 10Combi	31	9	n.s.

Table 69: Triangle test with the vinification BoBdyn and white wines of 2010

The statistical analysis of the ANOVA model revealed a most significant effect examiner as in previous years. The more precise the model can therefore calculate the operational and process effects or interactions.

3.4.1.2 Organoleptic Sensorial blind test

Resulted only in the criterion “minerality nose” had a significant difference between the methods. With a value of 36.1 (optimum 50) of the wine from the preparations treated plots was slightly better than that assessed from the Control plots with a value of 31.6. In the overall assessment no procedural difference was apparent. Only in Haut the wines differed slightly and in favour of the Control plot.

3.4.1.3 Sensitive Crystallisation of Wines

The results contain the scores given by Margarethe Chapelle to vitality, energy use and quality of the wine samples.

Parameter	Site	Treatment	Stage 1	Stage 2
Vitality	Elf	Combi	4	**6**
	Elf	Control	4	**4**
	Haut	Combi	7	**8**
	Haut	Control	9	**9**
	Auv	Combi	8	**7**
	Auv	Control	7	**6**
	Ech	Combi	8	**9**
	Ech	Control	5	**6**
Energy use	Elf	Combi	5	**7**
	Elf	Control	6	**4**
	Haut	Combi	5	**9**
	Haut	Control	9	**9**
	Auv	Combi	9	**6**
	Auv	Control	8	**7**
	Ech	Combi	9	**10**
	Ech	Control	4	**9**
Quality	Elf	Combi	4	**7**
	Elf	Control	4	**7**
	Haut	Combi	8	**10**
	Haut	Control	9	**9**
	Auv	Combi	10	**4**
	Auv	Control	9	**8**
	Ech	Combi	7	**10**
	Ech	Control	3	**9**

Table 70:Scoring of vitality, energy use and quality perceived in sensitive crystallisation images of 2010’s wines. Stage 2 is a judgement 24 h later than at Stage 1

Vitality

At Stage 1, vitality was higher at Auv and Ech. At Stage 2 vitality was higher in the Combi wines except at Haut.

Energy use

At Stage 1, energy use was higher for Combi wines in Auv and Ech. At Stage 2 energy efficiency of the Combi wines was higher compared to Control wines in Elf and Ech.

Quality

At Stage 1 quality scoring was higher in Combi at Auv and Ech; at Stage 2 in Haut and again in Ech.

3.5 Correlation

Here following the table correlations between leaf nutritional elements, SPAD and wood weight in the period 2009-2010, and three tables representing the correlations between SPAD and wood weight obtained from the statistical analyses of the single sites.

Correlation		SPAD	N	P	K	MgO	Ca	Bo	Fe	Mn	Zn	Cu	Ww
SPAD		1	,524**	-,771**	-0,199	,478**	0,066	,237*	0,018	-0,026	-0,195	-0,149	,425**
	Sig.		0,000	0,000	0,094	0,000	0,580	0,045	0,878	0,825	0,101	0,212	0,000
N		,524**	1	-,512**	0,005	-0,084	-0,098	-0,026	0,069	0,02	0,013	-,270*	0,189
	Sig.	0		0	0,966	0,485	0,412	0,829	0,564	0,865	0,914	0,022	0,112
P		-,771**	-,512**	1	0,05	-,308**	-0,074	0,052	-,316**	-0,117	0,2	,394**	-0,176
	Sig.	0	0		0,678	0,008	0,536	0,663	0,007	0,328	0,091	0,001	0,138
K		-0,199	0,005	0,05	1	-,438**	0,088	-0,027	0,116	0,058	-0,038	-0,067	-0,208
	Sig.	0,094	0,966	0,678		0	0,464	0,82	0,33	0,628	0,754	0,574	0,079
MgO		,478**	-0,084	-,308**	-,438**	1	,375**	,342**	0,043	0,144	-0,135	-0,124	,481**
	Sig.	0	0,485	0,008	0		0,001	0,003	0,722	0,229	0,259	0,3	0
Ca		0,066	-0,098	-0,074	0,088	,375**	1	,357**	0,024	0,161	-0,003	-0,044	0,225
	Sig.	0,58	0,412	0,536	0,464	0,001		0,002	0,842	0,176	0,978	0,713	0,057
Bo		,237*	-0,026	0,052	-0,027	,342**	,357**	1	-,445**	-0,091	0,137	0,174	0,22
	Sig.	0,045	0,829	0,663	0,82	0,003	0,002		0	0,446	0,25	0,143	0,064
Fe		0,018	0,069	-,316**	0,116	0,043	0,024	-,445**	1	,445**	-0,123	-,512**	-0,099
	Sig.	0,878	0,564	0,007	0,33	0,722	0,842	0		0	0,305	0	0,408
Mn		-0,026	0,02	-0,117	0,058	0,144	0,161	-0,091	,445**	1	0,197	-,444**	-0,006
	Sig.	0,825	0,865	0,328	0,628	0,229	0,176	0,446	0		0,097	0	0,963
Zn		-0,195	0,013	0,2	-0,038	-0,135	-0,003	0,137	-0,123	0,197	1	0,064	-0,048
	Sig.	0,101	0,914	0,091	0,754	0,259	0,978	0,25	0,305	0,097		0,596	0,686
Cu		-0,149	-,270*	,394**	-0,067	-0,124	-0,044	0,174	-,512**	-,444**	0,064	1	-0,056
	Sig.	0,212	0,022	0,001	0,574	0,3	0,713	0,143	0	0	0,596		0,642
Ww		,425**	0,189	-0,176	-0,208	,481**	0,225	0,22	-0,099	-0,006	-0,048	-0,056	1
	Sig.	0	0,112	0,138	0,079	0	0,057	0,064	0,408	0,963	0,686	0,642	

Figure 71: Correlation table for leaf analyses. Highlighted red when significant correlation and means are significant for p< .05 (*) F significant a 5% and p< .01(**) F sig at 1%. On blue when inversely propositional.

Correlation[a]		SPAD	Ww
SPAD	Corr. Pearson	1	,474*
	Sig.		0,019
	N	24	24
Ww	Corr. Pearson	,474*	1
	Sig.	0,019	
	N	24	24

Figure 72:Correlation table for SPAD and ood weight in single site Echandens

Correlation[a]		SPAD	Ww
SPAD	Pearson Corr.	1	,570**
	Sig.		0,004
	N	24	24
Ww	Pearson Corr.	,570**	1
	Sig.	0,004	
	N	24	24

Figure 73: Correlation table for SPAD and wood weight in single site Hauterive

Correlation[a]		SPAD	Ww
SPAD	Pearson Corr.	1	-0,276
	Sig.		0,192
	N	24	24
Ww	Pearson Corr.	-0,276	1
	Sig. (2-code)	0,192	
	N	24	24

Figure 74: Correlation table for SPAD and wood weight in single site Elfingen

As confirmed by the analyses carried out for the single sites one can notice a correlation highly significant between SPAD and wood weight. As well the trend of N and MgO increases with the increasing of SPAD. As confirmed in literature N contents and SPAD are dependant while P is inversely proportional.

Conclusions

Soil

The essential point of a biodynamic system is feeding the plant by feeding the soil. The theory underlying conventional farming is that of satisfying merely the chemical needs of the plants; the soil is considered only as an inert substrate. While the theory at the basis of biodynamic farming is the idea that all microorganisms, microinsects, earthworms, paedo fauna need to be activated because this confers to the soil a higher vitality and fertility which will fully provide for the plants' needs.

This study, as far as soil analysis is concerned, is dedicated to chemical analysis, analysis of the structure of aggregates, and that of microbial activity.

Soil analyses in 2013 show significant results on the soils treated with 500, here the K, Pw content was significantly reduced in all sites except for Elfingen. Also the humus percentage showed the same variance trend, however not in a such a significant degree. These peculiar results, contrarily to what found in literature, were confirmed in the differences observed comparing the soil analysis carried out in 2008 with those relative to 2013.

In this same period the humus percentage remained constant in the Control plot while it was reduced in the Combi plots, showing in the significant tests per site a high content in Elfingen.

The humus percentage and lower K and Pw present in the Combi plots and the interesting fact that only the Elfingen site (clay soil) showed higher values can be explained with the supply of organic matter peculiar to this site.

This lets us conclude that 500 is a probiotic and it needs organic matter in the soil in order to transform it for the production of humus. In fact while in Elfingen Biosol fertilizer was added to the treatments in the other 3 sites organic matter was not added (except for the small 3 t/He treatment in Hauterive). So treatment 500 consumed all the organic substance present on the soil causing a very poor and exhausted condition, proven by the analyses which evidenced a lack of macroelements in the soil that were mineralized by the microorganisms introduced with the 500 treatment. So it is important, in order to have good results with the 500 treatment to supply the soil with manure, green manure or biodynamic compost. To substantiated this thesis Elfingen showed the highest microbial activity; highly significant is the interaction Site per Depth of Cmic and Nmic which shows a higher activity in the superficial stratum (-0.2 cm) and a minor on in the 20/30 cm area. As supported by literature (reference)

Interaction site per position shows as well that the highest activity in Elfingen specially in the rhizosphere, while on the other sites the highest activity was proven to be in the middle of the strip.

The analyses carried out on the single sites evidenced in Hauterive a higher activity in the Combi plots than in the Control plots and in the superficial stratum. No results in the other sites.

The analysis of aggregate structure carried out with the FAL test has shown the statistical significance of Combi treatment on all sites. The most significant improvement is in Elfingen. This change of soil physical structure could be due to the increased microorganisms activity activated by the 500 treatment.

Plant

For the plant analyses were done the nutrients in leaf tissue, chlorophyll-index, phyto-alexine, disease pressure, and the weight and length of the shoots, no differences were observed in the nutrient analyses carried out on the leaves, except for Mn, Mg and K which had differences principally due to the region. Phosphorus was influenced by 501 per site and potassium from the interaction of 500 with 501 the same goes for Mn. It is difficult to outline the effect of the preparations, even though a reduction of the elements was observed.

No significant results obtained in the SPAD values but the nearly significant effect of 501 suggested to carry out a per site analysis.

The SPAD increases experienced with 501 and the extremely high values without 500, caused the plant to increase in weight (significant) and length (non significant). This can be explained in a stress of the plant that has more green leaves; the source is increased, however the sink as well.

In fact for the wood weight analysis on samples subjected to treatment with 500 or 501 an extension of the branches was observed in both cases. In the Combi plots a balance between vegetative and physiological function was achieved which is better.

The T 500 treatment tends to reduce length, and the wood weight shows a high statistical significance for the interaction 500 *site showing a lower weight in the 500 plots for three sites.

501 shows an impact on the wood weight of the shoot, the power, hence 501 lengthens a line of shoots, though it renders them fray at a vegetative level. 501 stresses them as they compete shooting upwards.

In 2008 was a difficult weather season and the incidence of disease particularly high. The interesting statistically high presence of phyto-alexine in the leaves of plants treated with 501 confirm the idea of a healthy status of plants to repair at the fungi attack. Keeping in mind that biodynamic is a prevention and not cure system, devised to help the plants to develop a better immune system and resist to disease attack. Remarkable but not statistically significant the treatment with 500; the combination of the two treatments yields a higher effect, confirming the totality of the method.

Grapes and Wine

No statistical differences were observed in the grape harvest yield and in the quality of the must concerning Oe', pH and acidity.

Wines for the period 2008-2010 were subjected to standard analysis and no statistical differences were found, except for lactic acid which was higher in Combi plot wines, while malic acid was lower in Combi plot wines. The degradation of this acid was complete in the biodynamic replication and, supposing a highest level of malic acid is easier for the bacteria to transform all the quantity. This suggests a better maturity status of the grapes at harvest, but these conclusion need a deeper oenological monitoring and analyses.

Wine sensorial analysis were done with a triangle test show no differences except for Chasselas 2009. In fact is difficult to say the better quality of a biodynamic wine, considering the high variables that change the taste of the final product depending on the practice adopted in the cellar.

The organoleptic sensorial blind tests evidence results only in the criteria minerality noses as parameter.

Wine Crystallization

Though the only information provided to the technician carrying the sensitive crystallisation analysis was that the wine samples came from a trial comparing biodynamic vs. organic, he found out information regarding the wine-making procedure. For most of the wines, the technician highlighted that they did not have an excess of sulphur (the chemical analysis showed levels of free SO_2 in the range of 15-35 mg/l for the 8 wines) he also stressed the calcareous influence in Auv sap, as well as in the wines from Auv, Haut and Ech, which completely agrees with the type of mother-rock present on the sites.

This study made it possible to analyise some farming parameters in different management and farming strategy examining biodynamic and organic agriculture with some significant results. However the data and the analysis collected was not always consistently replicated over the years and have not helped in a obtaining a clear understanding of specific phenomena but limiting only to the supply of some indications on the steps to follow in a future research.