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An Increase in Prominent Probiotics Represents the Major Change in the Gut Microbiota in Morbidly Obese Female Patients upon Bariatric Surgery

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Abstract: The global increase in obesity carries inherent health implications, with an increased BMI being a known risk factor for diseases such as type 2 diabetes, cardiovascular diseases, or different cancer types. The long-term effectiveness of diet therapy in addressing morbid obesity is extremely limited, with no adequate pharmaceutical agents available as treatment options, resulting in bariatric surgery being the only viable option to achieve and maintain significant long-term weight loss. Something that plays an important role in overall human health is the gut microbiome and its complex composition, which is usually altered and reduced in complexity/diversity in severely obese patients. In this study, the influence of bariatric surgery and the resulting weight loss on the gut microbiome composition of twelve morbidly obese (BMI $\geq$ 40) adult female central European patients was investigated by comparing the relative abundances of the major microbial phyla *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, and *Proteobacteria* pre- and post-surgery. We also aimed to give insight into the major changes in individual prominent and promising future probiotic bacteria characterized by an overall increase in abundance accompanied by a switch of enterotypes. Identifying specific microbial alterations associated with successful weight-loss outcomes may contribute to the development of future therapeutic interventions by supplementation with next-generation probiotics.

Keywords: gut microbiome; 16S rRNA sequencing; obesity; bariatric surgery; enterotypes

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1. Introduction

Obesity poses a significant public health challenge, resulting from changes in eating habits and disruptions in the body's regulation of energy intake, expenditure, and storage [1,2]. It has become a worldwide epidemic and a global public health crisis, with its prevalence tripling since 1975 in Western countries [3,4]. Furthermore, it was first classified as a disease by the World Health Organization in the year 2000 [5]. Unhealthy diets that are high in calories and sedentary lifestyles have been identified as key factors contributing to this widespread problem. However, the exact role of genetic, social, and environmental factors in the development of obesity remains not fully understood [3,6]. First, in 1963, the most severe form of obesity was described with the term "morbid obesity" [7], which is defined by a body mass index (BMI) of $\geq$35 kg/m^2 concomitant with significant comorbidities or a BMI of $\geq$40 kg/m^2 (Figure S1) [8,9]. The global rise in obesity carries inherent health implications, as elevated BMI is a well-established risk factor for diseases such as

type 2 diabetes [10], cardiovascular diseases [11], and various cancers like pancreatic, colon, or, exclusively in women, endometrial cancer [12]. Moreover, there is a concerning surge in the prevalence of metabolic disturbances associated with obesity in contemporary society, and this cluster of risk factors, referred to as metabolic syndrome, affects approximately one in four adults globally [6]. The projected increases in overweight and obesity among adults suggest that the burden of obesity-related morbidity and mortality will continue to rise in the coming decades, especially in combination with decreased physical activity.

The long-term effectiveness of diet therapy in addressing obesity is extremely limited, and currently, there are no truly adequate pharmaceutical agents available for the treatment of obesity, particularly in cases of morbid obesity [13]. Bariatric surgery represents the sole viable option for the most severe form of obesity, consistently achieving and maintaining significant weight loss. Since 1991, the National Institutes of Health (NIH) established guidelines for surgical therapy of morbid obesity, now recognized as bariatric surgery [14]. There are several bariatric surgery methods; the most abundant and established techniques in Germany are gastric bypass as well as sleeve gastrectomy (Figure 1). These standard techniques primarily function through a decreased stomach volume and malabsorption to delay the mixing of digestive juice with food [15].

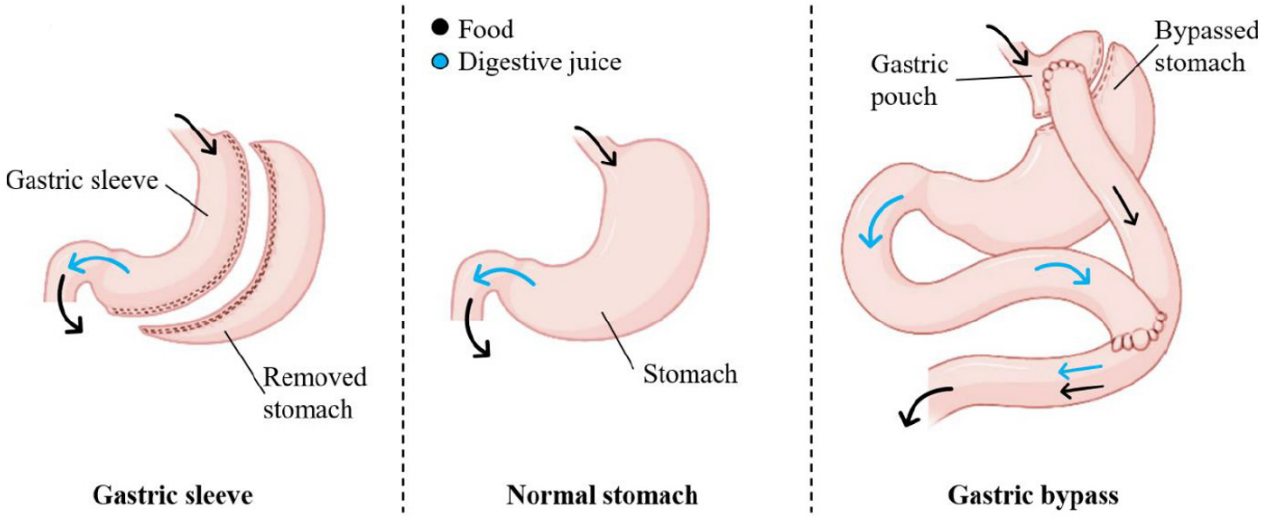

Figure 1. Comparison of stomachs and indicated direction in which the food is digested by the stomach (black) and the flow of the digestive juice (blue). (**Left**): Stomach after sleeve gastrectomy where ≥80% of the stomach is resected. (**Middle**): Stomach before bariatric surgery. (**Right**): Stomach after gastric bypass where the stomach is taken down right after the gastric inlet to create a gastric pouch so that the food bypasses a major part of the stomach.

The contribution of gut microbiota in the development of obesity has gained significant attention in the last few years. Research has demonstrated that the microbiomes of obese individuals exhibit structural and functional characteristics that contrast with their lean counterparts [16,17]. Preliminary evidence indicates that disruptions to the microbiome in obesity promote enhanced extraction of energy from food, leading to disturbances in nutrient distribution and the onset of obesity [18], which strongly suggests that the microbiome is a potential target for obesity-dedicated therapeutics. The human digestive system harbors trillions of microorganisms, making the gut microbiota highly diverse [19,20], and is recognized as one of the most densely inhabited microbial environments on Earth [21–23]. While the exact taxonomic composition that defines a "healthy" gut microbiome remains unclear, it is evident that microbial diversity is essential for maintaining the host's physical health. Obese individuals exhibit significantly lower bacterial diversity in comparison to their lean counterparts, and a reduction in fecal microbial gene richness is linked to various physiological indicators of obesity and metabolic syndrome [24–26]. Moreover, the aging

process plays a crucial role in metabolism, accompanied by changes in diet, medication, physical activity, and lifestyle, which in turn affect the gut microbiome, typically with a decrease in microbiome diversity over the years [27,28].

Elucidating the functional properties of the complex gut microbiome, often referred to as the black box of the human body, is still in its infancy. Ongoing metagenomic studies have revealed that most of the human gut microbiota consist of two predominant bacterial phyla, *Firmicutes* and *Bacteroidetes*, which together account for over 90% of the total community. Additionally, there are other less dominant phyla present in the gut, such as *Proteobacteria* and *Actinobacteria* [29–31]. It became clear that bariatric surgery and the resulting weight loss alter both the diversity and composition of the relative abundances of the colon phyla (expected changes during weight loss after bariatric surgery are displayed in Figure 2). This includes a decrease in *Firmicutes* as well as an increase in *Bacteroidetes* [32,33]. Several studies have discussed the evidence of an increased *Firmicutes*/*Bacteroidetes* (F/B) ratio as a marker of obesity, as, for example, a high level of *Firmicutes* is associated with a diet high in sugars, fats, starch, and proteins, as well as with a very efficient energy absorption from ingested calories [34,35]. In support of this assumption, it was shown that a low-calorie diet and the resulting weight loss result in the approximation of a normal state through a decrease in the F/B ratio. Likewise, it was also shown that *Bacteroidetes* are in general less efficient in extracting energy from food and are more abundant in the gut after high-fiber diets than previously [23,36–38].

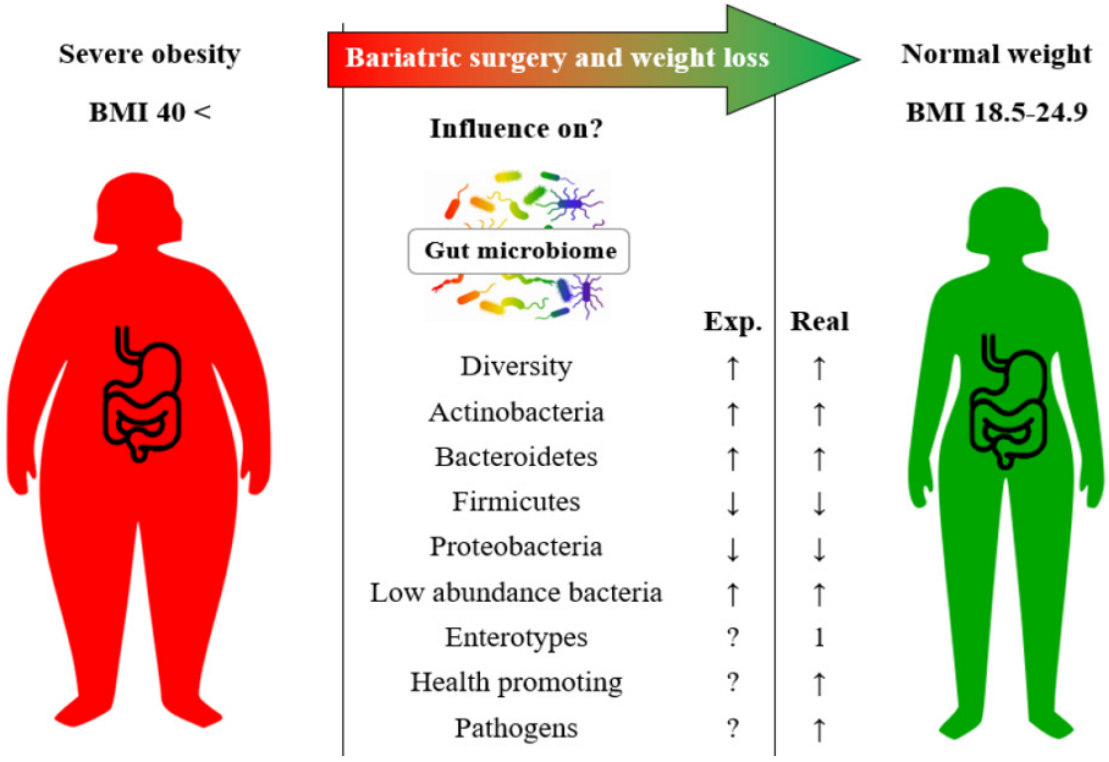

Figure 2. Expectations (Exp.) of the influence of weight reduction from morbid obesity (BMI > 40 kg/m^2) to normal weight (BMI 18.5–24.9 kg/m^2) through bariatric surgery on the human gut microbiome and other factors are listed. In addition to the expectations, the correlations found in our study (real) are shown as far as information can be provided; in the case of the enterotype, there was a change to mostly type 1. "?" denotes "unknown", "↓" denotes "low" and "↑" denotes "high".

The less dominant phylum *Actinobacteria* provides energy through their production of short-chain fatty acids (SCFA), like acetate, propionate, and butyrate, from carbohydrate fermentation. Also, they are crucial for maintaining gut barrier homeostasis and reaching higher abundances in healthy gut microbiome profiles [39–41]. In contrast, members

belonging to the phylum *Proteobacteria* are less abundant in the gut of healthy persons and increase while feeding a high-caloric diet [26,42]. In terms of determining diversity in the human gut microbial ecosystem, one possible parameter is the Shannon index [43]. Several studies observed a noteworthy negative association between the Shannon diversity index and obese people in contrast to lean counterparts [25,44,45] as well as an increase, and therefore a normalization, of alpha-diversity after bariatric surgery [46–48].

Contrary to the distribution at the phylum level, it has been suggested that variation at the genus level is discontinuous but forms three clusters known as enterotypes exhibiting varying proportional compositions. Humans in general like to cluster similar aspects into categories, which is also valid for gut microbiomes, and hence they were first namely introduced as enterotypes by Arumugam et al. in 2011 [49]. Diets rich in animal protein and fats, resembling a "Westernized" diet, were found to be associated with elevated levels of *Bacteroides* (enterotype 1), while diets high in simple carbohydrates, as commonly observed in agrarian societies, were linked to higher levels of *Prevotella* (enterotype 2). Whereas the drivers of enterotype 3 enriched in *Ruminococcus* are able to degrade mucins [8,49]. Additionally, it was discovered that the gut microbiota responded to short-term dietary interventions. However, it did not result in a shift in their respective enterotype tending to be highly stable [50,51].

The consumption of selected microbes, marketed as probiotics, has been well-documented as a potential method to influence gut microbiota. Probiotics are defined as live microorganisms that, when administered in sufficient quantities, confer a beneficial effect on the host's health [52]. It is important to note that the majority of probiotics available in the market primarily consist of microorganisms from the genera *Lactobacillus* and *Bifidobacterium* [53]. However, during the investigation of further potent probiotics, high abundances of *Akkermanisa muciniphila* were mentioned several times in connection with an improved metabolic status [54–56]. It also gained significant attention due to its verified positive correlation with health after bariatric surgery [57].

This study aims to investigate the impact of bariatric surgery and subsequent weight loss on the gut microbiome of twelve severely obese (BMI > 40 kg/m^2) female patients with various comorbidities. Beginning 6 months prior to surgery, the patients were monitored by an interdisciplinary team consisting of a surgeon, an internal medicine specialist/family doctor, a nutritionist, and a diabetologist. The goal was to lose 5–10% of body weight by the time of surgery. To achieve this goal, daily exercise and sports programs were intensified, and eating habits were changed towards a diet consisting of 30% fat, 30% protein, and 30% whole grains per meal. Additionally, food supplements were prescribed (high-quality protein powder and 150 vitamin supplements, including a complete A-Z multivitamin preparation, 2000 mg calcium, and 2000 I.U. vitamin D3, as well as vitamin E if no high-quality vegetable oil had been added to the diet). After bariatric surgery, a calorie-reduced diet, food supplements, and extra fluids were required. During the first 4 weeks after surgery, two protein shakes and one low-fat pureed meal were prescribed, after which the pureed meal could be discontinued. Types and doses of medication taken by the patients were monitored throughout this time. Although drug intake may have an influence on microbiome compositions in general, causing differences from patient to patient, this effect could be excluded here, since only the development of microbiome compositions in a given patient was regarded (delta of abundance). As medication for each patient was constantly applied in the same dosage before and after surgery, the observed differences resulted exclusively from weight loss upon surgery for the individual patients and not from differences in medication. Stool samples were collected from the patients both pre- and post-bariatric surgery (one day before and until 559 days after surgery), which involved either gastric sleeve or gastric bypass procedures. The patients' gut microbiome composition was analyzed using 16S rRNA next-generation sequencing and the data after surgery indicating the "patient's microbiome recovery" were compared to a healthy and young lean female, as well as to a male control volunteer. By examining changes in the gut microbiota following surgery, this study seeks to shed light on the potential microbial

contributions to the therapeutic effects of bariatric surgery and weight loss. Previous studies only focused on comparing weight loss after sleeve gastrectomy with the outcome after a very low-calorie diet (VLCD) over six months [58]. The aim was that both approaches, each involving five patients, would lead to significant changes in the gut microbiome composition and nutrient absorption. However, these studies had limitations, including the integration of patients with only the *Bacteroides* enterotype, the lack of comparison to control groups or a healthy cohort (except for SCFAs), and the limited number of patients included in the intestinal microbiome sequencing analysis. In contrast, our study addressed these limitations by including twelve morbidly obese female patients, as well as two lean controls (a young and healthy female and a male). We collected stool samples from the patients one day prior to surgery and at various timepoints thereafter, with the longest follow-up being 559 days. Additionally, we included patients with different enterotypes to provide a more comprehensive analysis. The overall outcome of our study suggests the need for new and more widespread studies in this field. By overcoming the limitations of previous research and providing a broader scope of investigation, our study serves as a valuable addition to the existing knowledge. Understanding how bariatric surgery influences the human intestinal microbiota can provide valuable insights into the complex interplay between the microbiome and obesity, also resulting in changes in immune cell populations of obese patients after bariatric surgery [59]. Furthermore, it may help to identify specific microbial markers or alterations associated with successful weight-loss outcomes, ultimately contributing to the development of future therapeutic interventions through the supplementation of probiotics for obesity and related comorbidities.

2. Results

In 1991, the NIH established guidelines for the surgical therapy of morbid obesity (BMI $\geq$ 40 kg/m^2 or BMI $\geq$ 35 kg/m^2 in the presence of significant comorbidities), now referred to as bariatric surgery, criteria which are also valid at the University Hospital Ulm [13,14]. Here, we present the results of the analysis of gut microbiota from patients with comorbidities like prediabetes, arterial hypertonia, depressive disorder, smoking, arthrosis, and/or hyperthyroidism (Table 1). Samples at timepoints after the bariatric surgery, with the shortest sampling interval being 122 days and the longest 559 days, and, for three patients, samples at intermediate timepoints, were collected and are shown in Figure 3. However, the results presented here exclusively refer to the latest timepoint for each patient (Figure 3). Age spanned from 27 to 65 years with most patients being between 40 and 50 years in age. The highest individual body weight before the surgery was 159 kg at a height of 162 cm, also resulting in the highest starting BMI of 61 kg/m^2 within this cohort (Table 1).

In order to eliminate potential differences in microbiome composition due to sex [60], we opted to include a healthy lean male volunteer (volunteer 2), along with a respective female person (volunteer 1), as controls. Both individuals are young, aged 27 and 32 years, with a BMI of 22 kg/m^2 (fitting in the "normal weight" criteria, as shown in Figure S1) and no comorbidities. Thus, they represent "the extreme" for the cohort of this study, thereby labeling the starting point of a microbiome on the way to its age-dependent alterations during the lifespan of an individuum.

Table 1. Overview of patients and lean controls. Assigned to their respective sample IDs (003-060, C1, C2) are gender, surgery type (S ≙ gastric sleeve, B ≙ gastric bypass), age, height, starting and end BMI, weight before and after bariatric surgery for patients or weight for lean controls, and pre-existing conditions and whether they smoke (Ex ≙ ex-smoker), indicated by checkmarks (✓). Pre-existing conditions (arterial hypertonia, hyperthyroidism, arthrosis, prediabetes, depressive disorder) are marked with a checkmark if they apply to the patients or lean controls.

S-ID	Sex	Surgery	Age [Years]	Height [cm]	Start BMI [kg/m^2]	Weight before Surgery [kg]	Weight after Surgery [kg]	End BMI [kg/m^2]	Arterial Hypertonia	Hyperth-yroidism	Arthrosis	Prediabetes	Depressive Disorder	Smoking
003	F	S	65	163	44	117	72	27	✓	✓	✓			
004	F	S	54	162	61	159	103	39	✓	✓	✓			
009	F	S	57	177	42	131	98	31	✓	✓	✓		✓	
012	F	S	30	158	47	118	70	28		✓	✓			
013	F	S	30	173	50	150	121	40	✓	✓	✓		✓	✓
016	F	S	39	163	41	109	75	28				✓		
022	F	S	55	173	49	147	89	30		✓				
023	F	S	27	167	41	114	72	26		✓	✓			✓
030	F	S	33	170	42	122	91	32			✓	✓		Ex
037	F	S	45	165	43	117	91	33		✓				
050	F	S	54	152	50	115	91	39	✓					
060	F	B	40	170	50	144	100	35		✓				
C1	F	-	27	158	22	56		22						
C2	M	-	32	179	22	70		22						

Background color for the last to lines indicate the control patients.

Figure 3. Timeline overview divided into quarters of all patients in this study including twelve patient-IDs (P-ID 003-060) and corresponding fecal sampling points. Day-1 describes the day prior to bariatric surgery for all patient samples. The shortest extraction point post-surgery was day 122 and the longest was day 559. In three patients, two stool samples were taken after surgery, whereby the respective last timepoints were included as post-operative in the following considerations.

In fact, all patients in this study experienced a decrease in body weight and BMI after surgery, as measured by the rate of BMI loss per week ("Delta BMI/week" in Figure 4), although the range of BMI reduction was broad, ranging from 0.2 to 0.6 BMI/week, with a calculated average value of 0.4 BMI/week across all patients. The BMI reductions defined as slow (ΔBMI < 0.3/week) and fast (ΔBMI > 0.3/week) were clustered into two groups of patients according to ages higher or below 50 years with the tendency for elder patients to exhibit slower weight loss compared to younger patients. Interestingly, exceptions existed in both groups with patient 022 being 55 years old and showing the fastest BMI reduction (−0.6 BMI/week) and patient 023 being the youngest at 27 years and achieving a BMI reduction close to the minimal change (ΔBMI = 0.2 BMI/week). Whereas patient 022 was characterized by an overall healthy constitution such as being a non-smoker with no arthrosis, patient 023 exhibited the extreme opposite, having both properties present.

The intestinal microbiome is composed of more than 1500 species, distributed across more than 50 different phyla [61]. Among these, *Bacteroidetes* and *Firmicutes* have been reported as the most dominant phyla, followed by *Proteobacteria*, *Fusobacteria*, *Tenericutes*, *Actinobacteria*, and *Verrucomicrobia*, constituting up to 90% of the total microbial population in humans [20,62].

The microbiome analyses presented here were conducted using 16S rRNA next-generation Illumina sequencing [63,64] of the respective stool samples collected both pre- and post-bariatric surgery, alongside control samples obtained from lean volunteers. High diversity, at least in the case of the gut microbiome, has generally been linked to overall health [65]. This association may be due to the enhanced functional redundancy, which can be achieved with a more diverse set of microbial communities, consequently leading to increased metabolic flexibility and adaptability [66]. A relative lack of diversity has been discussed as apparent in various diseases, including obesity [24,26,66].

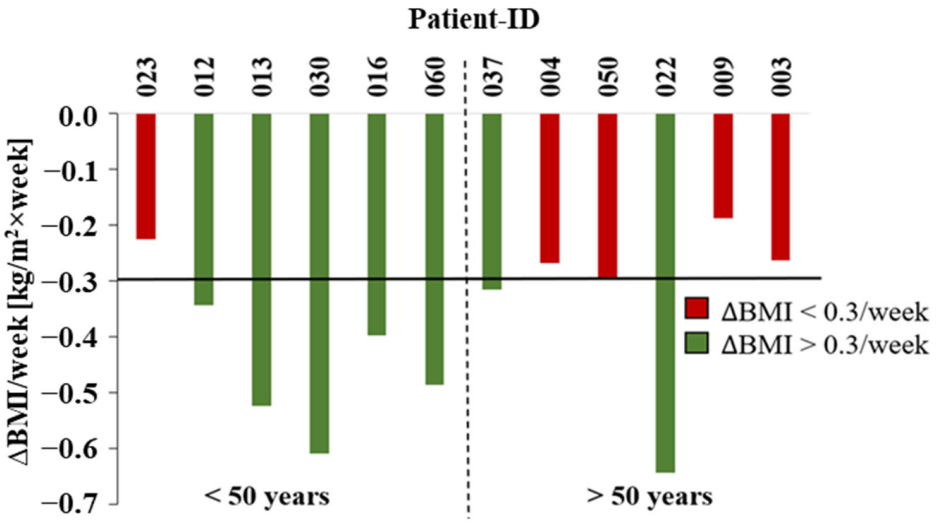

Figure 4. Changes in patient BMI (P-ID 003-060) per week ascending by age. Distinction between rapid decrease (ΔBMI > 0.3/week; green) and slow decrease (ΔBMI < 0.3/week; red). Shown are the clusters under 50 years and above.

The Shannon diversity index both takes richness (number of different taxa) and evenness (equal distribution of taxa) into account [43,67] and the values were compared before and after surgery, revealing a notable increase in diversity for nine out of eleven patients, extending from 3 to 42% (Figure 5). On average, the index experienced a 10% increase, rising from 6.7 to 7.4 after the surgery. Interestingly, a decrease in the Shannon diversity index was only observed in patients 012 and 013, who had the highest initial indices before surgery.

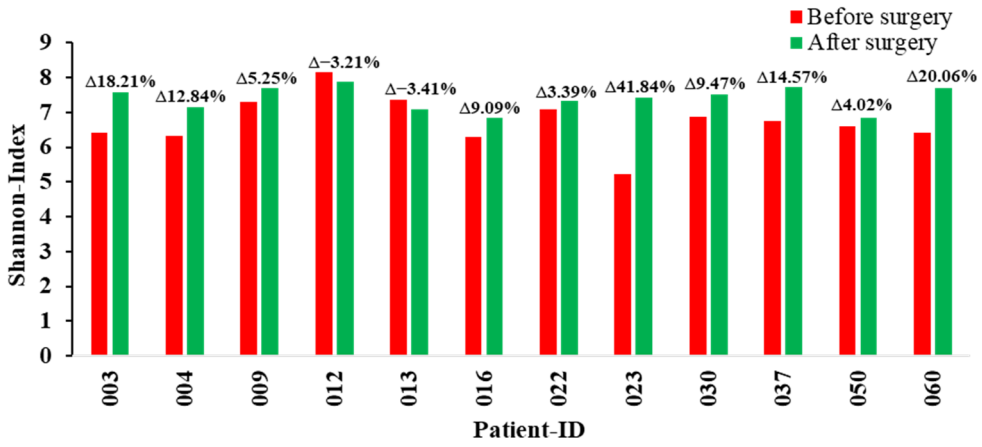

Figure 5. Alpha-diversity analysis of microbiomes of patients (P-ID 003-060) before (red columns) and after (green columns) bariatric surgery through the Shannon index. Above the paired columns, the percentage delta of the entropies is shown.

Major bacterial phyla in the human gut constituted the dominant groups, each accounting for more than 2% of the total relative abundance. Before the surgery, their relative abundances ranged from 7 to 49%, following the order *Actinobacteria* < *Proteobacteria* < *Bacteroidetes* < *Firmicutes*. Together, these phyla accounted for over 76% in total (Figures 6A and S2A–D). The remaining thirteen phyla, with abundances of each (drastically) lower than 2%, contributed to not more than 7.3% in total and were collectively grouped under the label "Phylum < 2%" (Figure S2F,G). Bacteria that could not distinctly be identified below their genus level as a consequence of the sequencing/analysis method constituted "the Unspecific" in our study (Figure S2E).

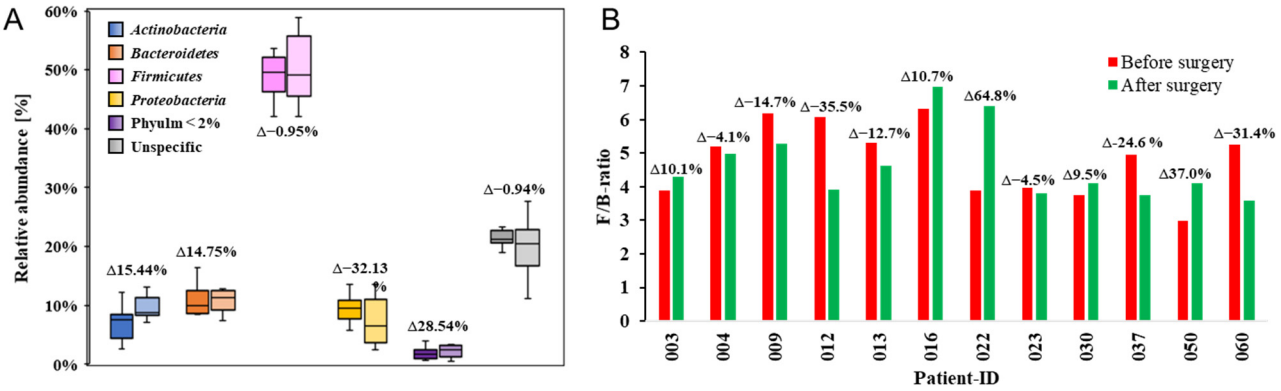

Figure 6. (**A**) Box–whisker plot of the major phylum groups *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, and *Proteobacteria*. Phylum < 2% and the unspecific bacteria of the patients (P-ID 003-060), as well as the percentage delta of each median, are shown above the box plot groups. The left boxes (bright colors) represent the relative abundance of the phylum before surgery and the right boxes (pale colors) show the values after bariatric surgery. (**B**) Ratio of the relative abundance of the gut microbiome phyla *Firmicutes* to *Bacteroidetes*. Depicted are data of patients before (green columns) and after (red columns) bariatric surgery (P-ID 003-060). The percentage difference in the F/B ratio of the patients from before and after bariatric surgery is shown above the columns.

A slight decrease of 0.95% in the median of the largest main phyla *Firmicutes*, as well as a significant increase of 14.75% in *Bacteroidetes* abundancy, was observed in the patients after surgery during their weight reduction (Figure 6A and Figure S2B,C). These findings align with a previous study investigating microbiome alterations upon changes in dietary behavior towards a low-calorie regimen [22,36].

Upon surgery, seven of twelve patients exhibited reductions in the F/B ratio between 4 and 36%. Interestingly, on the contrary, the F/B ratio increased by an average of 37.5% in five patients, with a maximum difference of 65% (Figure 6B), indicating an ambiguous pattern of these major bacterial groups after surgery within the cohort of individuals in our study.

In addition, *Actinobacteria* and the group labeled as "Phylum < 2%" gained higher abundances after surgery, with median values increasing by 15% and 29%, respectively. Conversely, *Proteobacteria* and "Unspecific" both decreased after surgery, with *Proteobacteria* assigning the most prominent difference (32%) of all phyla and "Unspecific" representing the lower end of alterations at only 1%. Generally, the scatter of values in the box–whisker plots was characterized by a broad distribution, probably indicating a considerable influence of individual parameters like (changes in) dietary composition, activity levels, the development of comorbidities, or personal lifestyle choices (e.g., smoking cessation, alcohol abstinence) after surgery.

In 2011, Arumugam et al. identified so-called enterotypes in human gut microbiomes, thereby introducing a system attempting to not only classify these types but also to describe the composition of microbiota with respect to their metabolic capabilities, in turn also taking personal preferences in the dietary composition into account for a more holistic view of nutrition, health, and the microbiome [49]. With seven individuals belonging to type 1, the *Bacteroides* enterotype was dominant before surgery in our cohort (Figure 6). The remaining individuals were distributed among enterotypes 2 and 3, with four members belonging to enterotype 3 and only one grouping into enterotype 2 (Figure 7, Table 1). This distribution demonstrates that even before surgery, the *Bacteroides* enterotype is dominant, while both others represent deviations from this standard. Microbiome enterotypes, per se, can be considered major systematic entities or biological markers not only for the classification of microbiomes but also for lifestyle and dietary behavior. Significant alterations in these parameters leading to the change from one enterotype to another may probably be the most significant hallmark for success in anti-obesity therapy in terms of microbiome integrity

and health. Notably, after surgery, the dominance of the type 1 enterotype extended almost to exclusiveness with only one patient left behind in her original type 3, whereas four patients switched their enterotypes, leading to the complete elimination of enterotype 2 in this cohort of patients. This remarkable major change also led to an approximation of the cohort to the lean control microbiomes, which both belong to type 1 (Figure 7, Table 2).

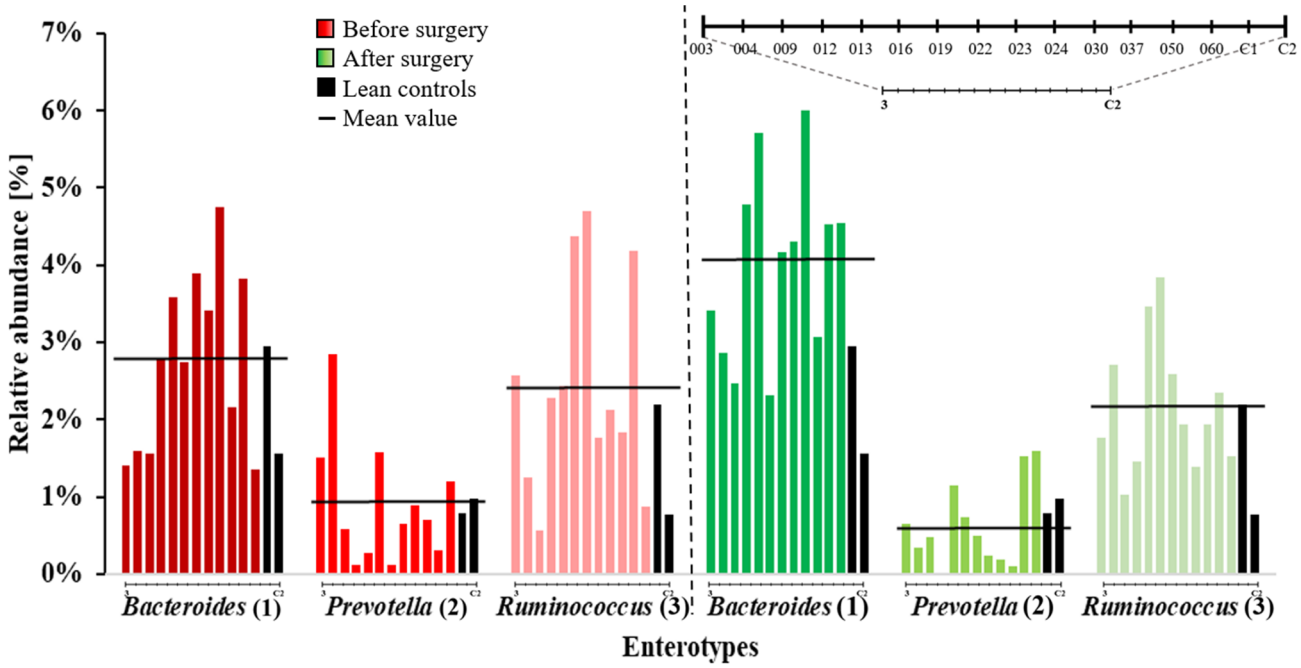

Figure 7. Associated relative abundance of enterotypes of patients (P-ID 003 to 060), as well as their respective values and lean controls (C1 and C2). The genera *Bacteroides* (enterotype 1), *Prevotella* (enterotype 2), and *Ruminococcus* (enterotype 3) are shown.

Table 2. Overview of sample IDs and respective enterotypes before and after patients' surgery (P-ID003-060) and the enterotypes of lean controls (C1 and C2). Changes in the enterotypes of the patients after surgery are indicated by checkmarks (✓).

S-ID	Before Surgery	After Surgery	Change in Enterotype
003	III	I	✓
004	II	I	✓
009	I	I	
012	I	I	
013	I	I	
016	III	III	
022	III	I	✓
023	I	I	
030	I	I	
037	I	I	
050	III	I	✓
060	I	I	
C1	I		
C2	I		

Background color for the last to lines indicate the control patients.

In addition to major taxa of relevance and higher systematic orders like the enterotypes, a vast amount of literature has accumulated over decades on individual genera of microorganisms with a suspected or proven significant impact on gut-related human health.

This includes already known prominent probiotic bacteria or strains currently emerging as promising candidates for future probiotics, as well as potent human pathogens (Figure 8A,B). We here have defined a group of health-related bacterial genera that exhibited changes in relative abundance in the patients' microbiomes. The members of this group showed alterations of at least 3% in both directions, either gain or loss, in the mean relative abundance within the respective microbiomes. This selected group accounts for approximately 10% of the mean relative abundance of all genera observed in the microbiomes of patients before and after surgery, as well as in the lean control volunteers.

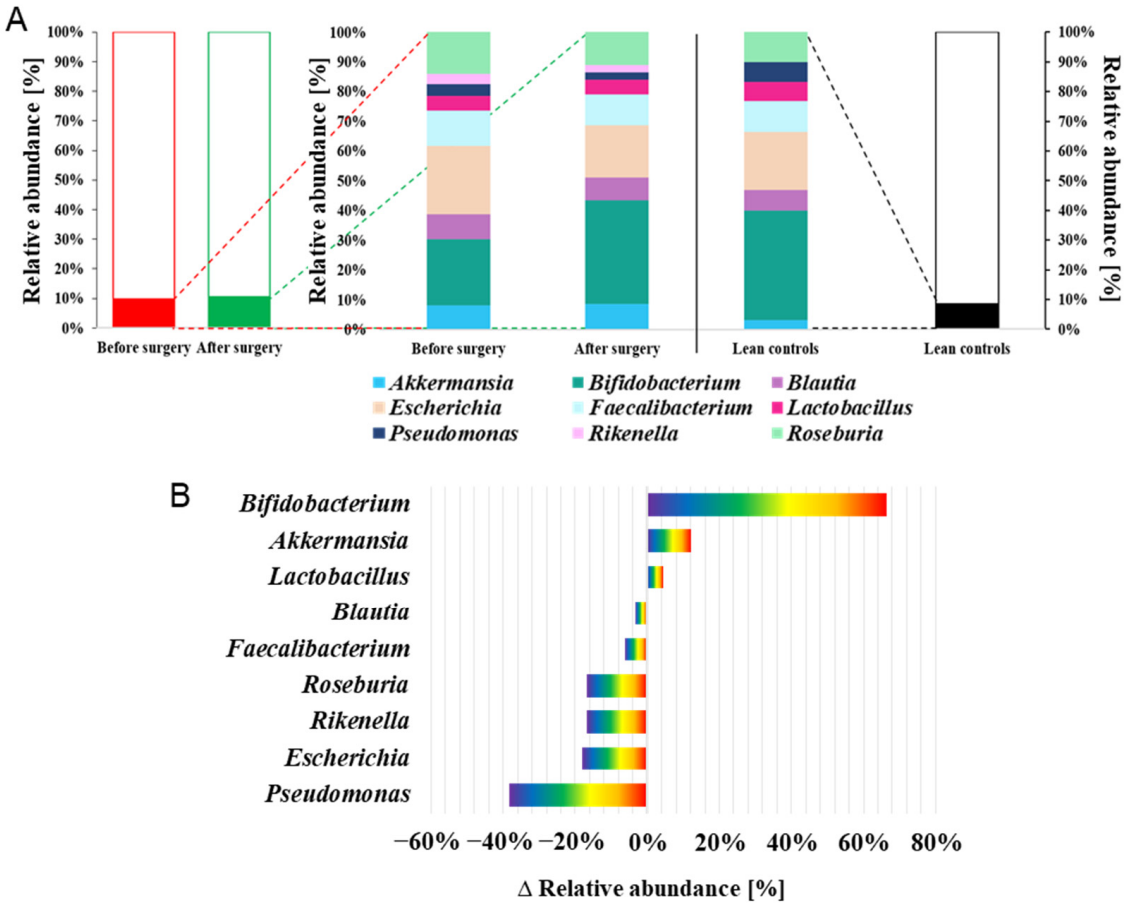

Figure 8. (**A**) Mean values of percent relative abundances in complete microbiomes of known health-related bacteria in patients (P-ID 003-060) before and after surgery, as well as in lean controls, are displayed. The group of known bacteria relevant to health consists of *Akkermansia, Bifidobacterium, Blautia, Escherichia, Faecalibacterium, Lactobacillus, Pseudomonas, Rikenella*, and *Roseburia*. (**B**) Changes (Δ) in the mean relative abundance of known health-related bacteria in patients sorted by greatest increase to greatest decrease from before and after surgery.

The largest effect (delta (Δ) relative abundance) of 66% was observed for *Bifidobacteria*, which are widely recognized and extensively discussed probiotic bacteria. They were drastically underrepresented before bariatric surgery but showed a substantial increase after surgery, not only approximating but even slightly surpassing the mean relative abundance within the healthy lean controls (Figure 8A). Compared to this, the second group of well-established probiotics, the *Lactobacilli*, only exhibited a marginal rise of 4% (Figure 8B). The positive development of the microbiomes, indicated by the increase in *Bifidobacteria*, appeared to be consolidated by a simultaneous increase in the genus *Akkermansia*. This includes *A. muciniphila*, which is at the moment frequently proclaimed as the upcoming next relevant probiotic [68] due to its association with a healthier metabolic status and better clinical outcomes following calorie restriction in overweight/obese adults [54]. The

opposite of the scale of alterations (i.e., decline) in relative abundance was marked by the genus *Pseudomonas*, which can include notable opportunistic human pathogens (e.g., *P. aeruginosa*), even completely disappearing from the microbiomes of four patients. A similar effect was observed for *Escherichia*, which were reduced after surgery, precisely meeting the mean values of this genus in the lean control healthy volunteers. Interestingly, the genus *Roseburia* considerably declined after surgery and weight loss by 17%, also strongly narrowing the gap with the abundance observed in the healthy control group (Figure 8A). Members of the genus *Roseburia* have been demonstrated to play a role in regulating gut barrier homeostasis [69], a function that may lose importance within the physiological background of a "healing" microbiome following bariatric surgery and weight reduction.

3. Discussion

Obesity has been recognized as one of the most important causes of ill health in Western countries because of poor diets and sedentary behaviors, resulting in an increased risk of comorbidities such as diabetes and/or cardiovascular disease. Changing nutrition to low-calorie diet concepts can reduce body weight, although these attempts are not sufficient for patients suffering from severe obesity and/or metabolic syndrome, since severely obese patients typically fail to persevere long-term dietary changes and the resulting productive and efficient calorie reduction. The ultima ratio for these individuals is bariatric surgery, nowadays widely accepted as the most efficacious and enduring treatment to enforce calorie reduction by limiting food ingestion [70].

In this study, the influence of bariatric surgery and the resulting weight loss on the gut microbiome of morbidly obese (BMI > 40 kg/m^2) female adult patients was investigated pre- and post-surgery by comparing the relative abundances of the major microbial phyla *Actinobacteria, Bacteroidetes, Firmicutes,* and *Proteobacteria,* low-abundance phyla of lower than 2%, and genera without further specification below the genus level, which were grouped together in the category "Unspecific".

The relative proportion of *Bacteroidetes* has been shown to be decreased in obese people in comparison with lean control volunteers, and this proportion can increase upon weight loss as a consequence of a low-calorie diet [22,36]. In the patients of our study cohort, *Firmicutes* and *Bacteroidetes* dominance was already present in the microbiomes before bariatric surgery and was also kept constant for the median relative abundance of *Firmicutes* after surgery, with a decrease of only < 1%. *Bacteroidetes* in comparison gained abundance after surgery with a drastic increase of 15%.

Alterations affecting the dominant phyla *Firmicutes* and *Bacteroidetes* were first described in obese animals and humans with increased abundances of *Firmicutes* at the expense of *Bacteroidetes* [22,35,36]. When these individuals were submitted to a calorie-restricted diet, an increase in their *Bacteroidetes* abundance was observed, as well as the normalization of their *Firmicutes*/*Bacteroidetes* ratio, in parallel with weight loss [22,36]. Support came from studies on animals kept on high-fat or high-fiber diets showing higher *Firmicutes* and *Bacteroidetes* abundances [23,37]. Similar findings were reported in children living in rural African areas, who consumed a traditional diet rich in fiber and showed higher proportions of *Bacteroidetes* and lower abundances of *Firmicutes* in the gut, compared to children from Western countries whose diet included large amounts of fat, sugar, protein, and starch [34,35,71]. These findings and results obtained from obese animals and humans [16,24,38,72–75] led to the suggestion that the *Firmicutes* are probably more effective in energy extraction from food than *Bacteroidetes*, thereby promoting more efficient absorption of calories and a subsequent gain in body weight and an increase in BMI [35,38].

A high F/B ratio has been discussed already in 2011 as a key property of obesity-associated non-healthy microbiomes [16,24], suggesting that the development of the mean F/B ratio after surgery observed with the patients presented here can be judged as a positive trend. In consequence, also due to associated fermentation activities, the occurrence of lower levels of *Firmicutes* could result in a reduction in energy harvest alongside caloric restriction and thus might benefit sustained weight loss and maintenance [58]. It has been reported

that *Bacteroidetes* produce less butyrate than *Firmicutes* but more acetate and propionate [76]. Butyrate is generally considered health-promoting due to its influence [77] on critical obesity-related parameters and comorbidities [78–80]. Propionate, however, stimulates the secretion of the obesity-related gut hormones glucagon-like peptide-1 (GLP-1) and peptide YY (PYY), leading to the inhibition of appetite [35,81]. The coexistence of *Bacteroidetes* and *Firmicutes* in the gut has been suspected to imply minimized competition for resources through cooperation or specialization, with a yet-unknown mechanism shifting this delicate balance towards *Firmicutes* [22,36]. In principle, the expected negative effects of a lack of reduction in the *Firmicutes* abundance may be compensated by the metabolic activities of the *Bacteroidetes*, leading to a behavioral optimization of the patients regarding food intake as a consequence of the physiologic interplay of fermentation products with the humoral system responsible for appetite control.

The median relative abundance of the phylum *Actinobacteria* was raised after surgery by the same amount as the *Bacteroidetes*—15%. *Actinobacteria* have been designated a relevant minority for the maintenance of gut homeostasis [41]. They also produce the carbohydrate fermentation products propionate and butyrate and, in addition, acetate, which are essential as a source of energy for epithelial cells [39,40]. Moreover, among them, *Bifidobacteria* have beneficial effects in the maintenance of the gut barrier based on their enormous capability to produce such fermentation end-products [82]. Acetate, for example, can protect the host from enteropathogenic infections such as entero-hemorrhagic *Escherichia coli* and *Shigella* [41,83]. Interestingly, *Bifidobacteria* in our study represented the genus with the strongest increase within the group of health-relevant bacteria, whereas the genera *Escherichia* and *Pseudomonas*, which both can contain important human pathogens, were drastically reduced. Without overinterpreting this coincidence in the changes in the relevant abundances of these prominent probiotics and pathogens, these developments suggest that bariatric surgery and the resulting weight loss may really tip the balance towards microbiomes of increased "healthiness". Another piece of evidence comes from the phylum *Proteobacteria*, which in our cohort of patients showed the most pronounced decrease in relative abundance. Apart from the fact that *Escherichia*, *Shigella*, *Pseudomonas*, and other Gram-negative pathogens belong to this phylum, in general, *Proteobacteria* have been described to be present in the gut of healthy humans at low abundances and elevated levels have been designated as a signature of microbial dysbiosis of the gut and even as a probably reliable biomarker [42]. As in our study, the major changes in the gut microbiome of animals and humans have been described to affect *Proteobacteria* independently from the host species, type of diet, and metabolic phenotypes before surgeries [2,84–86].

Apart from pathogens as certainly health-relevant bacteria and in addition to *Bifidobacteria* and *Lactobacilli* as prominent and long-term commercially available probiotics, several gut bacteria arising from studies on diseases as different as Alzheimer's disease, other neurodegenerative diseases, chronic intestinal inflammation (e.g., ulcus ulcerosa, inflammatory bowel disease, and Crohn's disease) are frequently suggested as promising next-generation probiotics [56,87]. *Roseburia*, especially *R. intestinalis*, has been associated with positive effects on inflammatory processes in the gut, presumably mostly due to its high butyrate production capability [88,89]. The fact that *Roseburia* showed a considerable reduction may be interpreted as indicative of a probable shortfall in the necessity of its anti-inflammatory metabolic benefits in the background of abundance adjustments by other bacteria for which it may compensate (e.g., *Bifidobacteria*). In contrast, the genus *Akkermansia* gained drastic relative abundance. The most eminent example, *A. muciniphila*, is characterized by its name-giving ability to degrade mucin residing in the gut of mammals, including humans [89,90]. The impact of *A. muciniphila* on health has been discussed in the context of different diseases including metabolic disorders such as obesity [55], diabetes mellitus [91], and neurodegenerative diseases such as multiple sclerosis [92,93], Alzheimer's [94], and Parkinson's disease [95]. In Alzheimer's disease mouse models, the development of symptoms is accompanied by a reduction in *A. muciniphila* in the gut microbiome [56]. A marker-like characteristic of microbiomes in obesity is a low abundance of *A. muciniphila* [57], and

upon weight loss, a restoration occurs, which is also the case in environmentally induced metabolic switches in animals [96]. *A. muciniphila* supplementation can be of therapeutic relevance in reducing body weight in obese humans [56].

One element of the human intestinal microbiome that can be influenced by diet is the enterotype, first introduced by Arumugam et al. in 2011 [49]. These states can be identified by their most prominent relative abundance of the genera *Bacteroides* (Enterotype 1), the *Prevotella*-enriched enterotype 2, and, in this study, the most viable *Ruminococcus* enterotype 3 [49]. Enterotype 1 enriched with *Bacteroides* was linked to a high protein and animal fat diet, whereas the *Prevotella* enterotype 2 was related to a diet with a great amount of carbohydrates [50]. Previous studies have demonstrated that these enterotypes tend to be relatively stable over extended periods of time. In an analysis based on two single collection points during the Human Microbiome Project [97], it was found that 84% of individuals did not switch their enterotype [51]. Over our cohort of twelve severely obese females, a broad variation in enterotypes can be observed, with seven patients belonging to the first enterotype, four clustering into enterotype 2, and only one into enterotype 3 before bariatric surgery. Furthermore, we revealed a significant number of four total changes in enterotypes post-surgery, divided into three alterations from the *Ruminococcus*-enriched enterotype 3 pre-surgery to enterotype 1 containing mostly *Bacteroides*, as is true for the control volunteers.

Our study represents the next important piece of evidence for understanding the development of the microbiome composition upon bariatric surgery and/or the corresponding weight loss. The term "and/or" in this context is not only correct but crucial since after years of small-scale studies with intrinsically extremely limited numbers of patients, it is still not possible to unequivocally distinguish between the causes and effects, or in other words, whether the changes in bacterial abundance distinctly result from surgery or weight loss. However, several properties of the post-surgery microbiome composition and the resulting changes behind it point in the direction of a normalization of conditions in healthy people. Interestingly, prominent and well-established as well as emerging future probiotics played major roles. The question of whether early supplementation with such present or next-generation probiotics can support or even prepone the desired "healing" of obesity-impaired microbiomes needs to be answered on the way to additional obesity therapies. However, this will require far more detailed and systematic studies with more patients and consequent monitoring not only of comorbidities but also strict control of the development of dietary behavior and lifestyle after surgery.

4. Materials and Methods

4.1. Study Cohort

The study included twelve adult ($\geq$18 years) female patients who underwent bariatric surgery in the Department of General and Visceral Surgery at Ulm University Hospital in the timespan from 2020 to 2022. Eleven out of twelve received a sleeve gastrectomy and one a bypass surgery. These patients also met the criteria outlined in the "German national S3-guideline for the surgical treatment of morbid obesity and metabolic diseases" (BMI $\geq$ 40 kg/m^2 with one or more obesity-associated comorbidities) [15]. Furthermore, patients with inflammatory bowel disease, systemic inflammatory disease, acute infections, cancer, autoimmune disease, or receiving immunosuppressive therapy were excluded from the study. The study participants included in the patient group were of non-Hispanic white ethnicity.

4.2. Anthropometric Measurements and Clinical Data

The body mass index (BMI) in kg/m^2 represents the ratio of an individual's weight in kilograms to the square of their height in meters. Prior to surgery and during follow-up examinations at respective timepoints at the Department of General and Visceral Surgery Ulm University Hospital, body weight and height were measured. The control group consisted of consenting volunteers ($\geq$18 years) with a BMI of $\leq$25.

4.3. 16S rDNA Next-Generation Sequencing

Fecal samples of twelve female patients before and after bariatric surgery, as well as fecal samples of two lean and young control volunteers (one female (volunteer 1), one male (volunteer 2)), were sent to BIOMES laboratory (Wildau, Germany) for bacterial abundance analysis using 16S rRNA next-generation sequencing. The sequencing was performed using INTEST.pro (Biomes Laboratory, Wildau, Germany) following the method by Lilja et al. [64]. In summary, microbial genomic DNA was extracted using a bead-beating technique, and the V3–V4 region of the 16S rRNA gene [63] was amplified and sequenced on the Illumina MiSeq platform using a 2 × 300 bp paired-end protocol (Illumina, San Diego, CA, USA). Normalized counts (abundance) were calculated by applying biological normalization of the copy number to the raw counts. The relative abundance was then normalized to 100%, and the resulting data space was transferred to a 0 to 1 value range [64]. The absolute and relative sequence counts for each taxonomical unit were provided.

5. Conclusions

The increase in severe obesity carries inherent health implications globally, with an increased BMI being a known risk factor for several diseases. In this study, the influence of bariatric surgery and the resulting weight loss on the gut microbiome composition was investigated by comparing the relative abundances of the major microbial phyla *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, and *Proteobacteria* pre- and post- bariatric surgery. We give insight into the major changes in individual prominent and promising future probiotic bacteria characterized by an overall increase in abundance accompanied by a switch of enterotypes. Identifying specific microbial changes associated with successful weight loss outcomes may contribute to the development of future therapeutic interventions through supplementation with next-generation probiotics.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/women4010007/s1, Figure S1: Classification of normal body weight and different stages of obesity by BMI-scale. The BMI is generally calculated according to the formula given in the "BMI"-field on the left. Figure S2: Relative abundance [%] of the identified major phyla of the gut microbiome, their respective medians, and the respective percentage delta. Data from patients (P-ID 003-060) before and after bariatric surgery, as well as controls (C1, C2), are displayed. (A) *Actinobacteria*, (B) *Bacteroidetes*, (C) *Firmicutes*, (D) *Proteobacteria*, (E) "Unspecific", (F) "Phylum < 2%". (G) Mean relative abundance [%] of the individual phyla summarized as "Phylum < 2%".

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